University of Alberta

The Effect of Broiler Genetic Strain and Parent Flock Age on Eggshell Conductance and Embryonic Metabolism

By

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Abstract

The effect of broiler strain (Ross 308 (R); Cobb 500 (C)) and parent flock age (Y-young, 29 wk; P-peak, R (34 -wk) and C(36 wk); PP-post peak, 40 wk; M-mature, 45 wk; O-old, 55 wk; and VO-very old, 59 wk) on eggshell conductance (G), and embryonic metabolism was examined. Data were analyzed by SAS® GLM at $P \leq 0.05$. Strain and flock age did not affect G. Strain did not affect average O_2 or CO_2 or heat output over the entire 21 d. Embryonic O_2 consumption (1, 7, 16, 17, 19, 20 d) CO_2 output (1, 2, 3, 4, 16, 17, 18, 19 d) and heat output (4, 7, 16, 17, 18, 19 d) differences existed between strains in early and late incubation periods. On average, embryos from Y, M, O and VO flocks produced more heat (88.33±2.14, 84.80±2.06, and 84.69±1.96 and 88.57±1.98 mW) than P (74.55±2.30mW) and PP flocks (76.06±1.95mW) over 21 d of incubation. The results indicate that both genetic strain and flock age influence embryonic metabolism but not G.

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DEDICATION

To my wife Amina, whose patience and belief in me was superb when I had to leave her in Ghana for these years in order to complete this program.

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CHAPTER 1: GENERAL INTRODUCTION

Changes in the Canadian Broiler Industry

In Canada chicken meat consumption has increased from 9.5 kg per capita in 1960 to 30.7 kg per capita in 2005 compared to beef and Pork meat which are the major meat products in the market which remained relatively stable or decreased from 31.7-31.9 kg and 25.9-22.9 kg respectively (Chicken Farmers of Canada, 2006). Thus, in 44yr chicken meat increased by 19% in Canada. Currently, there are about 2800 chicken farmers in Canada and in Alberta alone, the chicken producers increased from 240 to 285 between 1990 and 2005. In the same period, the total farm size in Alberta has increased from 253000 – 414000 kg live weight of birds. Because of current heath trends many Canadian consumers have higher interest for lean breast meat (Chicken Farmers of Canada, 2006). As a result, an increasing number of primary breeder companies are selecting birds for maximum breast meat yield.

According to data reported by the Economic Research Services of the United States Department of Agriculture, between 1925 and 1990 broiler age to market reduced from 15 to 6.5 wk. Over the same period, broiler weight increased from 1.3 kg to 2 kg. Because of these genetic improvements some physiological aspects of the broiler embryo such as the heart, respiratory organs and egg shell quality have been compromised (Ellendorff et al., 1995). Consequently, embryos of modern broiler strains consume more O_2 and grow excessively large in the egg creating an imbalance between their respiratory and metabolic rates (Burton et al. 2003). The result is that embryos of modern broiler genetic strains produce about 20% more heat during incubation than traditional breeds and have thus been compromised by then ability to survive

overheating (Boerjan, 2004).

When modern hatcheries were first built in about 20 yr ago, the incubators used were single stage. In single stage incubators, all the eggs are placed into an incubator at the same time, and are all removed and placed into hatchers at 18 d. In addition egg of only one embryo age a placed in the incubator. Hatchery manager could adjust incubator conditions any time to reduce stress put on embryos by heat (high or low temperature) and high or low humidity. In an effort to improve operation efficiency of incubators, the concept of multiple stage incubators was introduced. In these incubators eggs of various stages of embryonic development are incubated. The main reason for multiple stage incubation is to use the heat provided by the metabolism of older embryos to warm the eggs containing younger embryos and to increase the number eggs (also incubator eggs from different strains) and increase production. But the heat produced by embryos of modern strains is so higher that it places much more stress on the older embryos themselves than the younger embryos which result in increased late embryonic mortality. This higher mortality mostly due to the inability to remove excess heat produced by these embryos of modern genetic strains in multiple stage incubators. According to some hatcheries in Alberta this heat stress is especially critical during 16–18 d of incubation in the Cobb 500 strains than other strains. Because of overheating in multiple stage machines many hatcheries are going back to using simple stage machines in which temperature and air flows can be more strictly regulated.

While incubator design and function have a great part to play on embryonic, survival and chick quality, the selection of faster growing, larger broilers in the last 30 to 50 yr of breeding (Julian, 1998) has likely had a greater impact. Since the embryo

metabolism of these modern genetic strains has evolved, they may require different incubator conditions than strains of even a decade ago.

In Canada broiler breeder producers are paid on the basis of saleable chicks produced and any factor that can reduce chick quality or increase mortality will reduce efficiency in the broiler production chain. It is therefore important to assess these factors. In the continuing discussions previous studies on genetic selection and flock age and their link with factors that have been influencing the metabolism of chicken embryo will be highlighted. The goals of the discussion will also be used to develop two experiments to demonstrate how genetic selection and increasing broiler parent flock age in two modern genetic strains (Ross 308 and Cobb 500) has influence embryonic eggshell conductance, egg composition, embryonic metabolism, and ultimately the hatch chick and some chick components that can be use a measure of chick quality.

The Broiler Breeder Egg and Embryonic Development

Egg Formation. The egg is the container where the embryo spends its entire life. The reproductive system of the hen where the egg is formed is composed of an ovary and oviduct. The chicken has only one functional ovary and one oviduct. It is in the oviduct that all parts of the egg, except the yolk, are formed. The oviduct is divided into five distinct regions: infundibulum, magnum, isthmus, shell gland (or uterus), and vagina.

When the hen reaches sexual maturity, yolk formation begins as a gradual deposition of continuous layers of yolk material in the follicular sac (Romanoff, 1960). During incubation the embryo obtains the energy needed for development mainly from oxidation of yolk fat. Once the yolk follicle has reached maturity, it is ovulated and captured by the infundibulum, of the oviduct.

In the infundibulum, sperm if present may fertilize the female sex cell (visible as a small white disc on the surface of the yolk). The yolk quickly enters the magnum section of the oviduct where the dense portion of the albumen is added. The albumen consists mainly of about 10% proteins dissolved in water (as well as antibacteria properties) to provide additional nutrition for the growth of the embryo (Mickey et al., 1998). Unlike the egg yolk, it contains little fat but does provide additional nutrition for the embryo (Latour et al., 1998).

The next section of the oviduct, the isthmus, is smaller in diameter than the magnum. The inner and outer shell membranes formed in this region. The shell is added in the next section of the oviduct, the shell gland. The shell is composed mainly of calcium carbonate. It takes about 20 h for the egg shell to form. In the last portion of the oviduct, the vagina, a thin, protein coating called the cuticle is applied to the shell to help keep bacteria from entering the egg shell pores (Smith 1914). The egg passes through the oviduct small end first, but is laid large end first. In the vagina, the egg is turned horizontally just before laying and pushed out of the oviduct in a process called "oviposition" (Latour et al., 1998). As the egg is laid and cools down from hen's body temperature (41°C) to the environmental temperature. The inner shell membrane

contracts from the large end of the egg during egg cooling creating an air space between the inner and outer shell membrane.

Chick Embryonic Development. During egg formation embryonic development is occurring. Unless incubation temperatures (37.5°C) are provided after oviposition, further development of the embryo stops (Pattern and Calson, 1974). At oviposition, all blastoderm cells are alike, but as the embryo develops, cell differentiation and multiplication begin to occur (Smith, 1914). At 44 h of incubation, the heart and vascular systems join, and the heart begins to beat.

Two distinct circulatory systems are established; an embryonic system for the embryo and a vitelline system extending into the egg yolk which will provide the main energy source during development ((Baggot et al., 2001).). The development of these extra-embryonic membranes occur as temporal appendages that persist until the embryo can fully use its lungs later during incubation. These consist of the yolk sac, the amnion, the chorion, and the allantois. The chorion and allantois will fuse together to form the chorioallantois, an extension which encloses the albumen (Romanoff, 1960). The yolk sac is formed as an outer area of the blostoderm and it consists of three cell layers, which are in continuation with the cell layers of the embryonic disc (there is an ectodermal layer adjacent to the viteline membrane and endodermal layer adjacent to the yolk and in between them a layer of mesoderm cells). The yolk sac (which exist as the viteline membrane until d 4 of incubation) surround the yolk and its highly vascularized portion extends and receives absorbed nutrients from the yolk and transfer them to the embryo. Jensen (1969) as quoted by Baggot et al. (2001) noted that contact

of the embryonic tissue with viteline the membrane changes their structure and this enhances its rapture when yolk sac volume increases.

The amnion is formed from a layer of the endoderm and underlying avascular (does not develop blood vessels) mesoderm immediately close to the embryo. The appearance of muscles in the mesoderm allows the amnion to become contractile by 5 d of incubation But its blood cells arise from fusion of its avascular vessels with the vascular mesoderm from the allantois. The formation of a duct between the albumen and the amniotic sac called the seramniotic connection allows the movement of albumen protein into the amniotic fluid and subsequently into the yolk sac (Baggot et al., 2001). The Chorion develops from a tissue which continues with the amnion and away from the embryo. The allantois grows as a small bud from the primitive hudgut of the embryo and has both vascularized, join to the chorio-allantois, and avascular ends, join to the amnion, and supplies the blood systems of both the chorioallantois and the amnion (Baggot et al., 2001).. By 11 d of incubation, the allantois completely lined the inner portion of the chorion and the choriollantois forms and cover about 98% of the area of the eggshell membrane. The fusion acts as the primary respiratory surface especially during the second period of incubation. By the end of the 4^{th} d of incubation, the embryo has all organs needed to sustain life after hatching, and most of the embryonic parts can be identified (Pattern and Calson, 1974).

The embryo grows and develops rapidly during the first third of incubation through cell differentiation and formation of the vital organs. At this point the embryo depends largely on carbohydrate in yolk to obtain energy. The energy source being utilized by the embryo can be established by obtaining the respiratory quotient (RQ). In

the initial period of incubation the RQ is close to 1 which means the embryos is depending largely on carbohydrates and in the latter parts of incubation the RQ drops close to 0.6 which indicates that the embryos is metabolizing a lot of lipids. The RQ is calculated as the amount of O_2 consumed divided by the CO_2 produced by the embryo. Typical results from RQ data also confirm higher RQ figures during early incubation reflecting extensive use of carbohydrate sources rather than lipids. Barott (1937), as quoted by Kendeigh (1940), found that chicken embryos have an RQ of 1.0 at 2 d of incubation and an average RQ of 0.66 for the rest of incubation. This suggests the use of different substrates for metabolism during different periods of incubation. Further research confirmed the RQ to be between 0.9 and 1.0 in one to nine d old embryos, indicating utilization mainly of glucose (McKee et al., 1997) and for embryos close to hatching the RQ ranged from 0.72 to 0.74 (Hamdy et al., 1991) indicating utilization predominantly of fat.

By day seven, digits appear on the wings and feet, and the heart is completely enclosed in the thoracic cavity. Because major organ developments usually occur in the first third of incubation, unfavorable conditions such as low or high temperatures and heat stress could trigger malformations.

During the second third of incubation (8 – 14 d) the embryo grows in size. Between 8 to 9 d of incubation the metabolism is still quiet low, but between 10 and 14 d it rises steeply to reach a peak of about 600ml/d of O_2 consumption (Rahn et al., 1979). After 10 d of incubation, feathers and feather tracts are visible, and the beak hardens. In the last third of incubation (15 – 19 d) more body mass is added and embryonic metabolism and heat production continue to increase. At 18 d of incubation

O_2 consumption reaches a plateau where O_2 supply is insufficient to support the growing embryo (Pattern and Calson, 1974). This happens because the amount of air that can reach the embryo by simple diffusion across the eggshell is limited because of the fixed number of eggshell pores. The diffusive property of gases across the shell are influenced by shell thickness, pore diameter and length and the pore morphology (Brake et al, 1997).

Research shows that at the plateau stage of O_2 consumption O_2 utilization is directly dependent on the size of the embryo (Christensen et al., 2006). As a result, large embryos may die due to a lack of O_2 before they can initiate pulmonary respiration. During severe hypoxia both embryonic organ growth and function in the last phase of incubation may be affected (Christensen et al., 2006). Hypoxia occurs because supply of O_2 via simple diffusion across the eggshell is insufficient to support metabolism of the large embryo in addition to the extra energy required for the embryo to hatch. Embryos whose metabolic heat production increases after 18 d onward may become overheated. The heat stress can activate genes controlling heat stress proteins (Leandro1 et al., 2000) or cause hormonal imbalances that contribute to weak or low quality chicks (Decuypere and Bruggeman, 2006).

At about 19 d the chick penetrates the air cell (at the blunt end of the egg) with its beak in a process called internal pipping. After this point it draws in O_2 reserved in the air space to inflate its lungs to supplement the supply of O_2 by simple diffusion through the eggshell pores. There is gradual shift of the embryo from using the chorioallantoic membrane for respiration to pulmonary. During the process of internal pipping, the embryo uses carbohydrate store for energy rather than fat which requires

O_2 for ß-oxidation (Miller, 1998). As soon as the embryo pips into the air cell, it uses the drawn in O_2 to help provide the energy needed for the next stage of pipping out of the eggshell. In some embryos this O_2 may still not be sufficient to pass the embryo through the more difficult stage of breaking through the eggshell (external pipping) due to lowered O_2 partial pressure or tension. At 20 d of incubation, the chick is in the hatching position, the beak has externally pipped through the shell and the embryo is almost exclusively using the lungs for respiration.

After externally pipping out of the shell, the chorioallantois, which has served as the embryonic lung begins to dry up. The remaining yolk sac is drawn into the abdominal cavity of the embryo (Smith 1914). On hatching the chick becomes exhausted and rests, the navel opening heals, and the down feathers dry. Rahn et al. (1979) noted that by 21 day of incubation a 60 g egg will have taken up 6L of O_2 and given off 4.5L of CO_2 and 11L of water vapor.

Measuring Eggshell Conductance. The eggshell conductance is the process by which respiratory gases and moisture are exchanged by a diffusive process through the pores of the eggshell between atmospheric air and chorioallantoic capillary blood (Chiba et al., 2002). Tullet and Noble (1989) stressed that for accurate assessment of eggshell conductance, pore formation, shell thickness, total functional pore area, and passage of gas and water vapor through the shell should be taken into account.

One of the most commonly used methods for estimating eggshell conductance is shell thickness. The change in eggshell thickness with egg weight has been determined and these two relations used to predict the total functional pore area of the eggshell (Ar et al., 1974). Since shell thickness is negatively correlated with eggshell conductance, a

thick shell will have low eggshell conductance (Peebles and Brake, 1987).

Another method used to indirectly predict eggshell conductance is to measure specific gravity of the egg. Hamilton (1982) noted that since specific gravity increases with shell thickness, it can be used as an expression of the eggshell conductance constant. However, as good these methods may seem they only measure predictions of eggshell conductance and they do not consider how many total functional pores are present in either thin shelled eggs or thick shelled eggs.

One popular and simple method of assessing egg shell conductance has been extensively used by Rahn et al. (1979). In this method, freshly laid eggs are covered in desiccant and kept in a desiccator at a constant temperature and pressure for a maximum of 2 wk. Eggs are removed daily and weighed. Eggshell conductance values are calculated from daily egg moisture loss using the formula indicated below (Ar et al., 1974).

$$G_{H_2O} = \frac{M_{H_2O}}{\Delta P_{H_2O}}$$ Where

G_{H_2O} = water vapor conductance or eggshell conductance (mg/day/mmHg)

M_{H_2O} = the rate of water loss from egg (mg/day)

$\Delta P_{H_2O} = P_{H_2O} - P_o$

P_{H_2O} = water vapor pressure in the egg

P_O = water pressure outside the shell (desiccant = 0)

ΔP_{H_2O} = water vapor pressure difference across the shell which is equal to the saturated vapor pressure (mmHg).

Measuring Embryonic Metabolism. The egg is a closed system where the embryo grows and develops. The eggshell structure consists of the cuticle, the shell, an outer membrane and a fluid-loaded inner membrane. The fluid-loaded inner membrane initially prevents gaseous exchange with the outside environment and closes the system (Romanoff and Romanoff, 1949; Edwards et al., 2003). Embryonic O_2 consumption has been measured in closed respirometry systems, except for very large eggs (Vleck et al., 1980). In a closed system incoming air is not allowed to leave the system until all measurements have been taken. Closed-system respirometry gives only average values of the rates of energy metabolism over relatively extended time periods. In methods described by Vleck and Kenagy (1980), chicken eggs were placed in closed chambers and held at 37°C. At intervals throughout incubation air samples were taken from the chamber and injected into an O_2 analyzer to measure O_2 consumption.

In Laysan Albatross (*Diomedea immutabih*), Zhang and Whittow (1993), measured O_2 consumption and CO_2 production in eggs and hatchlings using a modified respirometer. The chambers containing the eggs were immersed in a water bath at 38°C and ventilated with air for 60 min before O_2 consumption and CO_2 produced were measured. In another study by Monge et al. (2000), eggs were individually placed in 10 cm x 6 cm Lucite chambers connected to a similar sized chamber by a water filled manometer. The two chambers were then submerged in a water bath maintained at 37±1°C. After a 0.5 - 1hr equilibrium period, the system was closed and 1 mL of O_2 injected with a syringe into the second chamber displacing the fluid in the manometer. The volume of air space in the manometer was used to determine how much O_2 the eggs consume as the water drops back to position.

The methods of measuring O_2 consumption discussed above have proved cumbersome and time consuming (O'Dea et al., 2004). Following these observations, Fasenko et al. (2004) and O'Dea et al. (2004) measured CO_2 production of chicken eggs and then indirectly calculated embryonic O_2 consumption by using the overall respiratory quotient of the chicken embryo throughout incubation (Romanoff, 1967). One advantage of using this system was that the systems more closely simulated industry hatchery conditions versus the previous methods which involved laboratory procedures. Though the system is simple, it still relied on the avian embryo's estimated overall respiratory coefficient of 0.78 for proteins, fats and carbohydrates to calculate embryonic O_2 consumption.

Recently, Mortola and Labbe (2005) have also measured oxygen consumption (VO_2) with an open-flow scheme in chicken embryos. This modified system by Mortola and Labbe, (2005) was earlier used by Frappell et al. (1992). The open flow system examines gas exchange of an entire egg mass and not a portion of the egg in its environment. In open flow method, an animal with a small metabolic body size can be examined in contrast to stop flow where a large animal is studied. In the method described by Mortola and Labbe (2005), gas flow was directed through respirometers and delivered by a pump under the control of a precision flow meter. The inflowing and outflowing gases were sampled, passed through a drying column, and monitored by a calibrated O_2 and CO_2 analyzer. In earlier argument MacFarlane and Frappell (2001), stated that equations used in the open flow system by Frappell et al. (1992) do not take into account the respiratory exchange ratio, i.e. the difference between the number moles of O_2 consumed to CO_2 produced.

In the above methods, though attempts were made to measure embryonic metabolism, there were a lot of limitations and they may have been used just set the stage for further steps into measuring embryo metabolism. In most of the methods, only one egg was monitored at a time and the number of reading that could be taken was limited. Also some method could not monitor the embryo till the end of incubation. Since they involved handling of eggs, thee= eggs could have become contaminated and alter actual results. Other methods made recordings tedious as the researchers had to keep constant watch on the apparatus.

The closest to measuring data accurately was the one demonstrated by O'Dea et al. (2004), but here too only CO_2 measurements could be taken to predict O_2 consumption and heat production. Relying on one RQ for the entire length of incubation may be misleading since the embryo uses different substrate during incubation. It has been shown that O_2 contributes about three folds into the heat production equation as CO_2 (Klieber, 1987) and therefore accurate measurement of the O_2 is much more important to accurately and reliably predict embryonic metabolism. It is important therefore that an embryonic metabolism system that can directly measure O_2 in addition to CO_2 in order to calculate embryonic metabolism in all 21 d of incubation is developed. In this regard embryonic system which will be more independent of human error once it is set up will be develop to fill up the gap in measuring embryonic metabolism.

Characteristics of a Hatching Egg

Egg composition of domestic fowl varies due to species, breed, age and nutrition. Generally, an egg contains approximately 64% albumen, 27% yolk and 9% shell and shell membranes (Marion et al., 1964). Recently analysis of the egg by Johnson and Ridlen (2006) show that the composition has changed slightly in modern chickens, with yolk making up 31%, 58% albumen while the shell and membranes make up 11%. The proportions of actual solids in each egg component and water were not separated in both egg measurements.

Baggott et al. (2002) recorded that egg albumen plays two major nutritional roles during development of avian embryo. First, the albumen provides water and an electrolyte for the embryo and, by the formation of sub-embryonic fluid, the embryo is able to relocate important nutrients within the yolk. Second, albumen proteins are an important component of embryonic and postnatal nutrition (Baggott et al., 2002). The yolk however is the major energy source during embryonic development and shortly after hatch. The yolk is predominantly made of fat with just 1% been carbohydrates and the fats are metabolized through the *B*-oxidation pathway during incubation to provide the needed energy for the embryo. The metabolism of the carbohydrates occurs during the early d of incubation and during the period of internal and external pipping. The yolk remains after hatching is used by the chick during the first few days after hatching.

The eggshell acts as a protective barrier between the egg and the surrounding environment. It also serves as the outer respiratory structure during embryonic life. The shell thickness and pore area are two features of avian eggs which must be thick enough to protect the embryo and egg contents, yet thin enough to permit the embryo to pip and

hatch successfully (Carey, 1986). The eggshell is mainly composed of calcium carbonate and the amount of shell that can be deposited during egg formation varies with flock age.

Effects of Albumen and Yolk Contents on Broiler Embryonic Development.

The importance of avian egg components in the determination of hatchling size and quality were investigated by Finkler et al. (1998). In the first experiment, 20% of the total albumen and yolk from chicken egg were removed during incubation to determine the effects of the components on body mass and various organ sizes. Removal of albumen resulted in a decrease in wet body mass corresponding to decreases in water content in the body and the yolk sac, and decreased tibiotarsus (shank) length. Removal of yolk had no effect on yolk free body mass, but caused decreases in both wet and dry yolk sac mass. In a second experiment by the same author, removal of 15% each of either egg components led to reductions in hatchling mass similar to the first experiment but it was the removal of albumen that caused the most significant reduction in hatchling weight. The authors explained that albumen, as the primary source of water in the egg, is the primary determinant of chick size and may influence hatchling success through size related limiting factors. While differences in yolk contents may influence neonatal quality as a nutritional supplement, they do not seem to result in greater tissue formation during the end of embryonic development (Finkler et al., 1998). Lourens and Meijerhof (2005) noted that after the embryo is hatched about 40% of the yolk remains unused and is absorbed by the newly hatched chick as residual yolk. If a chick has more residual yolk at hatch it means that it used less nutrients for growth and development than a chick with smaller amount of residual yolk (as long as egg size was the same).

Yolk Utilization, Yolk Free Body Mass, and Chick Length. Since the incubating egg is a closed system, during incubation the embryo develops entirely from the contents of the egg, using energy obtained mainly by oxidizing yolk fat. The distribution of energy in major components of a developing chicken egg as a function of embryonic age is shown in Figure 1. The figure show that the moment an egg is laid it contains approximately 80% yolk and only a minimal amount of it is used until d 7 of incubation. From this time onwards yolk utilization increases as embryo size increases. All the albumen is completely used (just before hatching the embryo ingests the albumen) but some yolk still remains at d 21. The largest proportion of lipid movement from the yolk to the developing embryo occurs during the last days of incubation when the yolk is completely drawn into the abdomen by the embryo (Ding and Lilburn, 2002). The quantity of residual yolk remaining after hatching is not as constant as is shown in Figure 1 in all chicks because embryo poorly utilize the yolk fat. It is expected that chicks with a large residual yolk will have a lower yolk-free body weight.

The relationships between post-hatch yolk-free bodyweight, residual yolk sac weight and chick length from a high yielding broiler breeder flock at 30 wks of age are shown in Figure 2. As shown in the figure, the amount of residual yolk varied between 1.5 and 6.5 g. Chick length was positively correlated with yolk-free body weight but negatively correlated with residual yolk weight. As yolk free body weight and chick length are related, it seems that chick length (from beak to toe in cm) is a good indicator of embryo development (Lourens and Meijerhof, 2005). It has also been determined that the weight of chicks at hatching are greatly influenced by the percentage yolk and percentage shell (Nestor et al., 1972).

Broiler Breeder Genetic Strain

Effect of Genetic Strain on Egg Weight, Yolk and Albumen Contents, Eggshell, and Yolk Free Body Weight. Selection is a viable way to increase egg dry matter, but simultaneously a change in the embryonic environment takes place (Hartmann et al., 2002).

In an experiment investigating the effects of broiler genetic strain of two modern broiler strains and a broiler that had not been selected since 1978, O'Dea et al. (2004) noticed higher yolk weight but lower albumen weight in the unselected strain compared to the two modern strains, though egg weights were not different. Eggshell contents did not differ among the three strains. When egg from the three broiler genetic strains were incubated, hatched chicks showed no difference in either yolk sac weight or yolk free body mass in all strains (O'Dea et al, 2004). In an earlier trial conducted in four strains, differences in genetic strain had a significant effect on the solids of whole egg, albumen, and yolk; however, the effect of genetic strain on yolk: albumen ratio was not significant (Ahn et al., 1997). Hartmann et al. (2003) found genetic correlations of 0.14, 0.76, 0.93, 0.14, and 0.99 between maternal effect on chick weight and the direct effect on yolk proportion, yolk weight, albumen weight, albumen dry matter, and egg weight, respectively. The heritability for yolk proportion, yolk weight, albumen weight, albumen dry matter concentration, and egg weight were 0.33, 0.43, 0.57, 0.38, and 0.60, respectively. They therefore concluded that breeding should be a useful way to increase egg dry matter such as albumen and it should not be expected that this will affect chick weight negatively. Because of these results it is expected that different genetic strains will have different proportions of egg components

Effect of Genetic Strain on Hatchability and Embryonic Mortality. Embryonic mortality is regarded as a direct fitness trait (traits that are influenced greatly by additive genetic effects) (Sewalem and Wilhelmson, 1999). Failure to hatch is due to infertility in some cases, while in other cases the zygote forms but fails to develop and eventually dies for a wide variety of reasons. In a cytological screening of 4,182 chick embryos from 10 strains and 5 strain crosses, the types and frequencies of chromosome abnormalities were reported. The study showed gross phenotypic effects, such as dead embryos, growth retardation and malformation which were noted to be mainly due to chromosome aberrations that occurred during genetic programs transferred to the offspring (Bloom, 1972).

Embryonic malpositions have been identified as a major cause of broiler embryonic deaths during late periods of incubation. In chickens, malpositions have been estimated to cause 50-55% of mortality in the last 3 d of incubation and 25% of total embryo mortality (Sanctuary, 1925). Research on chicken eggs has shown that eggs with easily distinguishable large and small ends have higher hatchability and a lower incidence of malpositions than do eggs with indistinguishable ends (rounder shape) (Benoff and Renden, 1980). Wilson et al. (2003) showed that slight variations in the incidence of malpositions in chicken embryos can be attributed to genetic strain.

Another major cause of embryonic death during early and late periods of egg incubation is the imbalance in metabolic activity of embryonic cells. During early incubation the disproportional growth of supply organs such as the heart and liver may result from a differential response to egg setting temperatures (Janke et al., 2002). Different cells from the same genetic strain may even have different physiological

response to temperature. While some cells respond quickly to incubation temperatures others may not initiate development causing damage to the development of some organs (Funk and Biellier, 1944).

In birds selected for growth, energy may be diverted to the development of tissues such as skeletal muscles in preference to the heart. This could lead to heart shock and early embryonic death (Lilja, 1983). Exposing chicken embryos to 10% hypoxia for 2, 4, and 6 h during 2 (control), 3, and 4 d of incubation showed that hypoxia has an impact on embryonic growth and impairs cardiac development at the time cardiac morphogenesis is taking place (Altimiras and Phu, 2000). The results showed that 4 d-vs 2 d-old embryos exposed to 6 h of hypoxia had an increased in mortality (38.9% in 4 d versus 18% for controls (2 d)). While only 8% of the controls displayed morphological abnormalities, 3 and 4 d old embryos exposed for 6 hr showed more frequent developmental abnormalities (25% and 30% respectively). The results suggest that acute hypoxic episodes do not have an impact when occurring very early in development (2 or 3 d). However, when the hypoxia occurs on d 4, survivability is decreased. This could also have a permanent effect on surviving embryos and ultimately affect the quality of the chick at hatching.

The major cause of death in late incubation can be traced to reduced O_2 tension and temperature effects of heat stress. Virtually little is known on the fatal effects of O_2 tension in chickens but in turkey Bagley and Christensen (1989) have attributed reduced embryonic heart metabolism to exposing embryos to lowered O_2 tension which is not favorable for embryos that are selected to grow longer. Hulet et al. (2003) noted that in different temperature treated eggs from a high-yielding broiler strain, late dead embryos

and pipped embryo deaths were significantly greater for higher temperature treated eggs compared to control group. They suggested that for the incubation of high-yielding broiler eggs, controlling eggshell temperatures during incubation could be important step to improving hatchability and post-hatch growth performance.

In the research in this thesis, O_2 consumption of several strains and parent flock ages over a 21 d incubation period will be studied. The results will indicate if embryos of eggs from different sources require more or less O_2.

Effects of Genetic Strain on Eggshell Conductance. Broiler breeders are selected for growth and better feed conversion with much lower regard for traits such as shell conductance. As a result modern broilers consume less feed and grow very fast. Usually when primary breeder companies select for layers the eggshell is taken into consideration but because the selection for broilers has focused on meat, the broiler breeder has lost some beneficial egg traits especially relating to the eggshell strength. It has been stated already that the thickness of eggshell can affect gas exchange between the egg and its surroundings (Peebles and Brake, 1987). This is because if the shell is too thick, pore length increases which limit gas diffusion through the shell. If the shell is too thin a lot of moisture will be lost from the egg which can affect embryonic development and weight. O'Dea et al. (2004) using eggs of equal weights found that genetics does not affect eggshell conductance. Christensen and Nestor (1994) also reported that selection for growth or egg production had only slight influence on eggshell conductance in turkeys. The current research will examine the conductacenof two strains which have not been compared before.

Effects of Genetic Strain on Embryonic Metabolism and Growth. Fasenko and Robinson (2000) found differences in heart and hepatic weights in hatchlings of three different broilers genetic lines studied. Further to this it was reported that longer internal and external pipping times in one line may be the cause of higher late embryonic mortality (Fasenko and Robinson, 2000).

Blood thyroid hormone concentrations, which help the body use energy, stay warm, and keep the brain, heart, muscles, and other organs working as they should is reported to differ in two genetically different chicken lines (Christensen et al., 1995). Christensen et al. (1995) compared embryos from a modern Arbor Acres genetic line developed from larger eggs with more albumen and less yolk, and a random bred control population (Athens Canadian random bred line). The study showed that the modern broiler embryos possessed greater ratios of triiodothyronine (T_3) to thyroxine (T_4) than the random bred embryos at external pipping and hatching. The authors argued that this situation may arise because chick embryos between modern and random bred genetic lines can have different carbohydrate metabolism during the later periods of development.

In a similar report by Tona et al. (2004) the T_3 and T_4 ratios were again significantly higher in standard heavy broiler strain embryos compared to two experimental lines carrying dwarf genes. In addition, embryonic P_{CO_2} and heat production were also higher in the standard broiler lines than the dwarf lines suggesting different metabolic rate among the lines. Earlier, Tona et al. (2003a, b) had reported that embryos from strains with higher levels of these physiological indicators can have better post hatch growth.

In some avian species, selection has influenced the pattern of embryonic development with additional energy being diverted to increase the weight of the gastrointestinal tract and the chorioallantois (Lilja and Olsson, 1987). Strains selected for different purposes show different metabolic strategies during the incubation period and at hatching.

Embryos of modern high yielding strains have been shown to produce much more heat during incubation than the more classical type of birds (Hulet and Meijerhof, 2001). The consequence of this was that the internal egg temperature (embryo temperature) of modern high yielding strain embryos was higher than the classical strains. It was argued that unless incubator conditions are adjusted in these instances, more embryonic mortality can result. Many commercial hatchery managers are leveling the incubator turning racks from 16 d onwards to improve air flow and remove the excess embryonic heat to reduce mortality.

Factors that influence O_2 consumption, CO_2 production, metabolic water production and heat production, also influence yolk utilization and embryonic development. Embryo temperature is the result of the balance between heat input and heat output. Besides increased CO_2 production, more metabolic heat is produced when more yolk is used for growth and development. This extra metabolic heat should be removed from the eggs to prevent the embryo from overheating.

In the research conducted in this thesis, it is expected that the strain that has been selected for high breast muscle yield (Cobb) will have a higher metabolism than the strain selected for the whole bird market (Ross).

Broiler Parent Flock Age

Effects of Flock Age on Egg Weight, Yolk and Albumen Contents, Eggshell, and Yolk Free Body Weight. A significant curvilinear effect of flock age on yolk weight has been reported by Suarez et al., (1997). This implies that as flock age increases, weight of yolk can increase twice as much in the egg older flocks compared the yolk present in a very young flock egg. In results provided by Ar and Meir (2002) they indicated that there was an increased albumen fraction while yolk fraction decreased with increased egg weight and flock age. In other studies it has been found that as flock age increases the yolk weight increase (Peebles et al., 2001) while the albumen weight decreases (Ahn et al., 1997). It is also contended that, as flocks age and egg weight increases, shell thickness decreases (Flectcher et al., 1981; Scottish Agricultural College, 2001). Though the weight of hatching eggs can influence broiler live performance regardless of hen age, egg composition is altered with egg weight, but such alterations do not seem to have major effects on hatched broiler chick growth and processing yields. Alterations in egg weight and composition do not affect the proportion of yolk sac to body weight particularly with eggs from very young hens (Vieira and Moran, 1999).

Effects of Flock Age on Hatchability and Embryonic Mortality. Broiler hatching eggs from two young (30 and 31 wk) or two old (52 and 53 wk) breeder flocks were investigated in two experiments (Elibol et al., 2002). Both hatchability of total eggs and hatch of fertile eggs decreased as flock age increased in both experiments. The results also indicated higher embryonic mortality in 52 and 53 wk old flock than 30 and 31 wk old flocks. Though all experimental eggs were stored for 3, 7 or 14 d before

incubation hatchability and mortality were always better in the younger flock parent flocks (Elibol et al., 2002).Embryo metabolism of the different flock ages may play a role in embryonic survival.

Effects of Flock Age on Eggshell Conductance. Reports indicate that as flock age increases egg size and shell conductance increases probably due to an increase in pore numbers (Scottish Agricultural College, 2001). Rahn et al. (1974) found that gas movement across bigger eggs is higher due to the increased number of pores; hence these large eggs lose more moisture and have higher eggshell conductance. Young hens are known to produce eggs with thicker shells, longer pores and lower conductance than older hens (Peebles and Brake, 1987). The eggshell thins with age, but may thicken in very old flocks while eggshell pore diameter may decrease (Peebles and Brake, 1987). Correspondingly, the eggshell conductance lowers at the beginning and at the very tail end of laying period (Peebles and Brake, 1987) when hatchability is often poorest (Brake et al., 1997). Recent results have shown that eggs from 37 wk old flocks had higher conductance than eggs from 45 or 53 wk old flocks, which did not differ from one another (O'Dea et al., 2004).

The O_2 demand of embryos prior to pipping is greater in larger eggs than it is in smaller ones due to embryo size. However, if gas conductance is increased, the O_2 requirement of the embryos will be met but this will also increase moisture loss and the potential for dehydration. Similarly a lower gas conductance may induce hypoxia and death. In addition, the embryo may be poisoned by its own CO_2, or drown in excess metabolic water (Rahn et al., 1974). There is a need, therefore, to maximize O_2 conductance but have balance with other factors.

The conflicting results regarding flock age and eggshell conductance necessitate that this be further examined.

Effects of Flock Age on Embryonic Metabolism and Growth. In determining embryonic metabolism in eggs from three broiler breeder strains at 33 and 38 wk, O'Dea et al. (2004) showed that embryos from 38 wk old flocks had higher embryonic metabolism than 33 wk old flocks over the entire incubation period. These results were independent of egg weight as egg weight was held constant. The effects of broiler breeder age (26, 28, and 30 wk) on embryonic growth between 16 and 21 d of incubation, embryo and chick body composition and chick weight, were evaluated by Peebles et al. (2001). Results of this study showed that broiler chick body weight at 21 d was lower for 26 wk old breeders compared to those at 28 and 30 wk. Embryo moisture content was found to be lower at 16, 17, and 19 d of incubation in eggs from breeders at 26 wk when compared to those at 28 wk of age. Reidy et al. (1998) suggested that although nutrient utilization in all avian embryos may follow a fairly well and similar established sequence of events embryos from different avian age groups may be different in their metabolism of various lipid fractions.

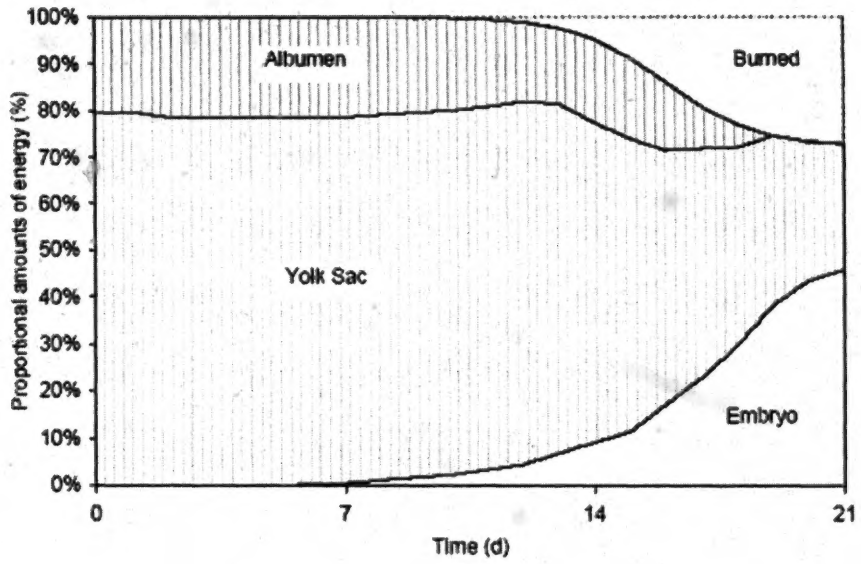

Figure 1: Distribution of energy in major components of the developing chicken embryo as a function of age (Romanoff, 1967)

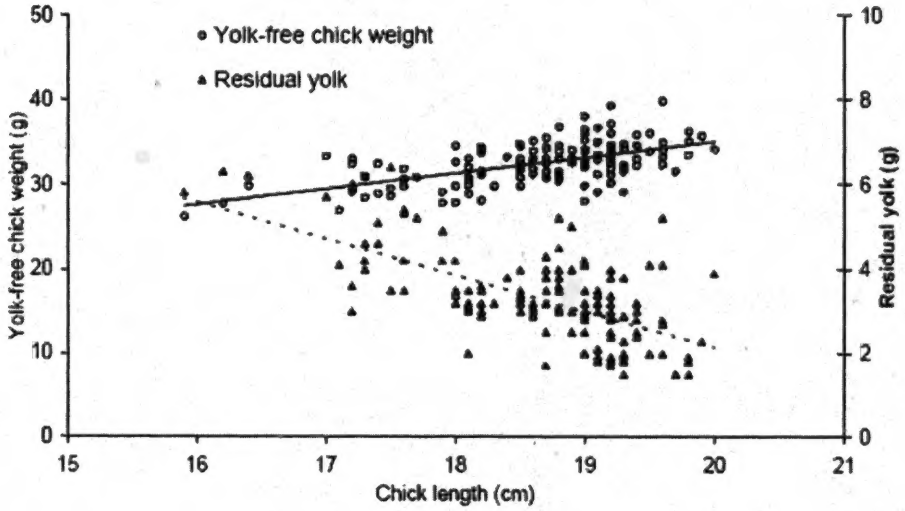

Figure 2: Relationship between yolk–free chick weight, residual yolk, and chick length (Lourens and Meijerhof, 2005)

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CHAPTER 2: *Experiment 1: The Effects of Broiler Breeder Genetic Strain and Broiler Parent Flock Age on Eggshell Conductance*

INTRODUCTION

During normal incubation bird eggs continuously lose water. The amount and rate of water loss is directly proportional to egg size but rate of loss is inversely proportional to incubation length (Rahn et al., 1976). Thus bigger egg will loss a lot of moisture and as the incubation time lengthen, the total moisture loss also increases (Rahn et al., 1979). In a study with sooty terns, it was argued that irrespectively of egg a hatching egg would lose about 12-15% of its initial weight prior to pipping and in the process the volume of water lost is replaced by an equivalent gas volume in the air cell, which provides O_2 for initial inflation of the embryonic lungs (Visschedijk, 1968).

Conductance is a measure of the ease with which moisture and gases diffuse across the pores of the shell. Gas exchange across the shell is limited by the diffusive properties of the gases themselves and by the functional properties of the eggshell such as the pores and shell thickness, and shell membrane permeability (Ar et al., 1974). These factors are fixed when an egg is formed. Water vapor and gas transport across the eggshell takes place by molecular diffusion (Wagensteen et al., 1971).

For the majority of incubation, diffusion of O_2 across the shell is sufficient to sustain the needs of the embryo. However, in the latter stages of incubation, as the embryo reaches pipping, its metabolism increases in proportion to its size, but the functional egg pores remain the same (Rahn, 1981). This means that the O2 provided by diffusion across the shell is not sufficient to sustain the embryo. In order to survive, the embryo must pip into the air cell and initiate pulmonary respiration.

Selection in the past three to five decades has dramatically changed the growth potential of broilers. The size of the broiler eggs has also changed in proportion to broiler size. Modern broiler breeders lay bigger eggs as compared to strains of 30 yr ago. Selection for broiler growth has increased the physical egg characteristics (egg weight, volume, surface area) but adjustments have not been made to increase the functional characteristics (conductance) to make up for increased embryo size and embryonic metabolism associated with the larger egg size (Christensen and Nestor, 1994). With genetic selection the eggshell characteristics including pore size, pore morphology, pore geometry and porosity and eggshell conductance have not increased (Ar et al., 1974) and this has likely to O_2 limitation in the growing broiler embryo during the plateau stage of O_2 consumption (Wineland et al., 2006).

By investigating eggshell conductance differences in modern and unselected domestic chicken strains at different flock ages, O'Dea et al. (2004) indicated that genetic strain has not affected eggshell conductance, but eggs from 37-wk-old parent flocks had higher conductance than eggs from 45 or 53 wk old parent flocks. On the contrary, Rahn et al. (1979) consistently found that eggshell conductance increased with the size of the egg though this comparison was done with egg from different species of birds. However egg size was found to be positively correlated with age, and as flock age increases the size of the egg also increases (Shanawany, 1984). Rahn et al (1979) determined that, especially bigger eggs, the O_2 demand of the embryos prior to internal pipping was greater than in smaller eggs. However, eggshell conductance did not increase in proportion to the egg mass and this limited O_2 consumption in the bigger eggs at pipping and thus increased incubation time.

Wineland et al. (2006) investigated eggs from two broiler strains known to have either low or high eggshell conductance. The eggs were exposed to different O_2 and temperature regimes. From 18 d of incubation (defined by the authors as the beginning of the plateau stage of O_2 consumption) they found that elevated temperature and depressed O_2 in the incubator retarded intestinal maturation especially in low conductance eggs. When O_2 levels were lowered, there was a reduced maltase activity in low conductance eggs compared with high conductance eggs from 18 to 21 d. The above study showed that a reduced ability to metabolize carbohydrates may also limit post-hatch survival and growth.

Eggshell conductance studies from most research have concentrated on differences between unselected and modern genetic broiler strains and also other species of poultry. Rahn et al (1979) determined in 90 species and 15 orders of birds studied that the eggshell conductance consistently increased with egg size. Data presented by O'Dea (2004) compared to previous eggshell conductance data in the chicken (Ar et al., 1974) show that modern chicken strains have higher conductance than strains investigated 40 yr ago. Shafey (2002) reported that genetic make up of birds and egg size (also affected by flock age) influence eggshell characteristics. The type of bird (heavy meat type Hybro or small leghorn type) influences the optimum egg size and eggshell conductance for successful hatching. The objectives of the current research was to determine if broiler genetic strain and parent flock age influence eggshell conductance and the major egg component characteristics; yolk, shell and albumen in two modern genetic strains (Cobb 500 and Ross 308). The hypothesis is that Cobb 500 will have higher eggshell conductance than Ross 308.

MATERIALS AND METHODS

Location and Periods of Research

All experiments were conducted at the Alberta Hatching Egg Producers Hatchery at the University of Alberta in Edmonton. The city of Edmonton lies within latitude 53° 34' N and longitude 113° 31' W and is 668 m or 2,192 ft above sea level with atmospheric pressure at the experimental site varying between 92 and 95 kPa.

Experiments started on 18th October 2005 and ended on 1st July 2006. All experimental procedures including the handling of eggs and chicks were approved by the Faculty of Agriculture, Forestry and Home Economics Animal Policy and Welfare Committee according to the principles and guidelines outlined by the Canadian Council of Animal Care (1993).

Experimental Materials. Six trials were conducted using two broiler breeder genetic strains (Ross 308 – which has been selected to meet broad market requirement and Cobb 500 – known for its high breast meat yield). Each trial was conducted to compare the two strains at the same time

Broiler breeders were grouped into six age categories (Young (Y), Peak (P), Post peak (PP), Mature (M), Old (O), and Very Old (VO) corresponding to 29, 34 or 36, 40, 45, 55, 59 wk old flocks respectively. The same flocks were used for all but P research trial. Each age category represented a trial. All eggs were obtained from Maple Leaf Poultry, Wetaskiwin, Alberta.

Experimental Design and Eggshell Conductance. In each of the six trials, 15 eggs each from the two strains were selected within a close weight range (64.4 – 64.8g). Although the egg weights in each trial were selected within a close weight range, the increase in average egg size as flock age increased, and the genetic differences between the strains required that the average weights of the eggs be adjusted (±0.1g) between trials.

Fifteen eggs per strain were weighed and placed vertically in a 300 ml plastic container as described by O'Dea et al. (2004). The eggs were completely covered with desiccant[1] and then the containers placed in a desiccator[2]. The desiccator consisted of an airtight metal box frame measuring (30 cm X 30 cm X 26 cm) with glass panesl. To equalize the air pressure between the inside of the desiccator and the external environment, the desiccator was modified with 30 cm of plastic tubing. A 1 cm hole was drilled in the right upper corner of the desiccator and the 30 cm long plastic tubing was inserted. The tubing had an inner diameter of 0.79 cm and was packed with desiccant.

The edges of the tube inserted into the desiccator were sealed with silicone caulking to prevent air leakage. The exposed end of the tube was then covered with rubber mesh to ensure that air flowed through the tube freely while also preventing the desiccant from falling out of the tubing. An electronic thermometer/hygrometer was placed inside the desiccator to record daily temperature and relative humidity. Due to equalized pressure between the inside and outside of the desiccator, desiccator temperature was equivalent to the room temperature.

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[1]Drierite: W. A. Hammond Drierite Company Ltd., Xenia, OH.
[2]Despatch V Series Model VRC2-26-1E, Minneapolis, MN. 55044

The eggs were removed and weighed daily (at the same time of the day) for 9 d to determine the amount of moisture loss from the eggs. Desiccator temperature was also recorded daily and expressed as saturated vapor pressure of the air.

Eggshell Conductance. The water vapor conductance of the egg or eggshell conductance was calculated using the formula given by Ar et al. (1974).

$$G_{H_2O} = \frac{M_{H_2O}}{\Delta P_{H_2O}}$$ Where

G_{H_2O} = water vapor conductance or eggshell conductance (mg/day/mmHg)

M_{H_2O} = the rate of water loss from egg (mg/day)

$\Delta P_{H_2O} = P_{H_2O} - P_o$

P_{H_2O} = water vapor pressure in the egg

P_O = water pressure outside the shell (desiccant = 0)

ΔP_{H_2O} = water vapor pressure difference across the shell which is equal to the saturated vapor pressure (mmHg).

During the first 2 d that the eggs were placed in the desiccator, temperature recordings were not used in the calculations of saturated vapor pressure. This period was considered an equilibrium period between the desiccator and the room conditions especially temperature and pressure.

Egg Composition Characteristics. At the end of 9 d all eggs were removed from the desiccator and weighed and then broken open and the yolk separated from the albumen. The yolk and the albumen were poured into a separating funnel. The albumens been almost water drained from the funnel into a beaker while the yolk remains in the funnel. The yolk and the shell were placed in a pre-weighed aluminum petri-dish and reweighed together with their contents. Wet albumen weight was calculated by subtracting the yolk and eggshell weights from the whole egg weight at 9 d. The eggshells and yolks were then placed in a drying oven[2] at 65^0C for 4 d and then reweighed to determine their dry matter composition. All the egg components were expressed as percentage of the total fresh egg weight.

Statistical Analysis

Data was analyzed as a 2 X 6 factorial design, using the General Linear Models (GLM) Procedure of SAS® (SAS, 2005). The GLM was used as there was no random variable as studied to provide a random error factor apart from the error occurring in trials to test main effects. Compared to ANOVA, it was appropriate because of some missing data as a result of broken yolks during separation from the albumen. The Comparisons of eggshell conductance and egg compositions between strains and among parent flock ages were made by the lsmeans method at $P < 0.05$ and differences between lsmeans separated by the pdif procedure of SAS. Again the lsmeans was sued due to some missing data to find the smallest means for any two comparisons. Unless otherwise specified, values are reported as lsmeans ± standard error of the means.

RESULTS AND DISCUSSION

Egg Weight and Eggshell Conductance.

Effect of Breeder Strain. As shown in Table 1, breeder strain did not influence initial fresh egg weight. This was expected since the eggs were selected to be very near the same weight. Final egg weight (after eggs were in the dessicator 9 d) and eggshell conductance did not differ significantly between the two strains investigated, indicating that the shell properties were not influenced by broiler strains. This was also the case when different strains with the same egg weight were examined by O'Dea et al. (2004).

Effect of Breeder Flock Age. Initial egg weight was significantly lower in eggs obtained from Young (Y) breeder parents by about (0.5 g) compared to other breeder parent ages. This was the case, even though effort was made to select eggs that had the same average weight at each flock age. Although the lower egg weight observed in the Y flocks was statistically different it is unlikely that such a small difference would have any biological effect. Final egg weight and eggshell conductance were not influenced by flock age. This finding was somewhat surprising considering that the percentage dry shell did vary with flock age (Table 2). However the main factor may be that egg weights were kept the same irrespective of flock age. This result is different from other reports showing that that as flock age increase eggshell conductance increases or decreases (Rahn et al., 1979; O'Dea et al., 2004).

Effect of Breeder Strain and Breeder Flock Age Interactions. From Table 1, the results show that there were some influences of the interaction between broiler genetic strain and parent flock age on initial and final egg weights but ultimately eggshell conductance was not significantly influenced.

Egg Component Characteristics

Effect of Breeder Strain. Table 2 shows egg component weights expressed as a percentage of fresh egg weight between the two genetic strains. There were no effects of genetic strain on any of the parameters measured. This was due to the fact that initial egg weight between the two strains was selected within a close range.

Effect of Breeder Flock Age. The oldest flock age (VO) had a significantly higher percentage wet yolk weight compared to the younger parent flocks (Y, P, and PP) (Table 2). This trend of increasing yolk as flock age increased was also evident for percentage dry yolk. This was expected since it is known that yolk weight increases as flocks age (Peebles et al., 2000).

In contrast, wet egg albumen content decreased with increasing breeder flock age. This has been demonstrated by Ahn et al. (1997) who showed that the lowest yolk:albumen ratio is in eggs produced by 28 wk old hens and the highest ratio is in 55 and 78 wk old hens. Peebles et al. (2000) also found significantly higher yolk:albumen ratios as parent flock aged

Contrary to the yolk contents, as the breeder flocks age increased, percentage wet and dry shell decreased (Table 2). This was an anticipated result as previous research has shown the weight of shell decreases as the flock age increases (Fletcher et al., 1981).

Effect of Breeder Strain and Breeder Flock Age Interactions. The results from Table 2 show there were no significant effects of breeder strain and flock age interactions on any of the egg characteristics measured.

Summary and Conclusions

In summary, even though it is recognized that the parent flocks and broilers of R vs C have different physical characteristics studied, the current results show that no difference in eggshell conductance exist between the two strains or the six flock ages studied. However there were significant differences in yolk, shell and albumen fractions with respect to increasing breeder flock age.

Industry observations show that the C strain has larger eggs than the R strain when eggs from the same parent flocks ages are obtained. By investigating eggs of equal size, the current research eliminated any physical differences between the two strains due to differences in egg weight. Future research designs should use eggs of average weight for each strain at a particular flock age and calculate conductance as a percentage of fresh egg weight.

The current research showed that as flocks aged, eggshell weight decreased. With the reduction in shell weight it was expected that conductance would increase due

to a reduction in shell thickness and pore length. The results do not support this hypothesis. Future research should examine in detail the physical properties of the egg that affect conductance such as eggshell thickness and number of pores in modern genetic strain.

Table 1: Effect of two broiler breeder genetic strains and six breeder flock ages on egg weight and eggshell conductance.

Source	Initial egg weight[1] (g)	Final egg weight[2] (g)	Eggshell conductance (mg/d/mmHg)[3]
Genetic strain			
Ross	64.61 ± 0.03 [a] (90)[4]	61.65 ± 0.07 [a] (88)	19.15 ± 0.39 [a] (88)
Cobb	64.54 ± 0.03 [a] (90)	61.60 ± 0.07 [a] (88)	18.40 ± 0.39 [a] (88)
Flock age			
Young	64.17 ± 0.06 [b] (30)	61.66 ± 0.11 [a] (30)	19.36 ± 0.68 [a] (30)
Peak	64.63 ± 0.06 [a] (30)	61.58 ± 0.11 [a] (29)	18.06 ± 0.69 [a] (29)
Post peak	64.65 ± 0.06 [a] (30)	61.56 ± 0.11 [a] (30)	18.39 ± 0.68 [a] (30)
Mature	64.65 ± 0.06 [a] (30)	61.89 ± 0.11 [a] (28)	17.46 ± 0.71 [a] (28)
Old	64.69 ± 0.06 [a] (30)	61.44 ± 0.11 [a] (29)	20.20 ± 0.69 [a] (29)
Very Old	64.66 ± 0.06 [a] (30)	61.64 ± 0.11 [a] (30)	19.17 ± 0.68 [a] (30)
Interactions			
Ross * Young	64.48 ± 0.08 [b] (15)	62.01 ± 0.16 [a] (15)	19.13 ± 0.97 [a] (15)
Ross * Peak	64.56 ± 0.08 [a] (15)	61.59 ± 0.16 [ab] (14)	17.52 ± 1.00 [a] (14)
Ross * Post peak	64.66 ± 0.08 [a] (15)	61.54 ± 0.16 [bc] (15)	18.52 ± 0.97 [a] (15)
Ross * Mature	64.64 ± 0.08 [a] (15)	61.86 ± 0.16 [a] (14)	17.57 ± 1.00 [a] (14)
Ross * Old	64.66 ± 0.08 [a] (15)	61.24 ± 0.16 [b] (15)	21.24 ± 0.97 [a] (15)
Ross * Very Old	64.67 ± 0.08 [a] (15)	61.37 ± 0.16 [b] (15)	20.92 ± 0.97 [a] (15)
Cobb * Young	63.85 ± 0.08 [a] (15)	61.31 ± 0.16 [b] (15)	19.59 ± 0.97 [a] (15)
Cobb * Peak	64.70 ± 0.08 [a] (15)	61.56 ± 0.16 [ab] (15)	18.61 ± 0.97 [a] (15)
Cobb * Post peak	64.64 ± 0.08 [a] (15)	61.58 ± 0.16 [ab] (15)	18.26 ± 0.97 [a] (15)
Cobb * Mature	64.66 ± 0.08 [a] (15)	61.93 ± 0.16 [a] (14)	17.35 ± 1.00 [a] (14)
Cobb * Old	64.73 ± 0.08 [a] (15)	61.64 ± 0.16 [ab] (14)	19.16 ± 1.00 [a] (14)
Cobb * Very Old	64.66 ± 0.08 [a] (15)	61.91 ± 0.16 [a] (15)	17.43 ± 0.97 [a] (15)

[a-c], columnar means with different superscripts differ significantly ($P \leq 0.05$).
[1] Initial egg weight = Egg weight at the time of collection.
[2] Final egg weight = Egg weight after 9 d of being covered in desiccant.
[3] Eggshell Conductance = Rate of moisture loss / saturated vapor pressure.
[4] (n) = sample size.

Table 2: Effect of two broiler breeder genetic strain and six breeder flock age on egg yolk, shell and albumen characteristics

Source	% wet yolk[1]	% wet shell[2]	% dry yolk[3]	% dry shell[4]	% wet albumen[5]
Genetic strain					
Ross	30.78 ± 0.25 [a] (75)[6]	8.79 ± 0.06 [a] (89)	16.36 ± 0.21 [a] (74)	8.27 ± 0.07[a] (89)	55.77 ± 0.27 [a] (74)
Cobb	30.54 ± 0.24 [a] (84)	8.84 ± 0.06 [a] (88)	16.21 ± 0.19 [a] (84)	8.26 ± 0.07 [a] (88)	56.18 ± 0.25 [a] (84)
Flock age					
Young	25.80 ± 0.42 [d] (27)	8.67 ± 0.11 [ab] (30)	14.15 ± 0.34 [c] (27)	8.62 ± 0.11 [ab] (30)	61.70 ± 0.43 [a] (27)
Peak	29.94 ± 0.46 [c] (23)	8.71 ± 0.11 [ab] (30)	15.33 ± 0.37 [b] (23)	8.46 ± 0.11 [b] (30)	56.49 ± 0.50 [b] (23)
Post peak	30.93 ± 0.43 [bc] (26)	9.03 ± 0.11 [a] (30)	15.47 ± 0.34 [b] (26)	8.98 ± 0.11 [a] (30)	55.37 ± 0.44 [bc] (26)
Mature	32.15 ± 0.43 [ab] (26)	9.01 ± 0.11 [a] (28)	18.14 ± 0.35 [a] (25)	8.96 ± 0.11 [a] (28)	54.59 ± 0.44 [cd] (25)
Old	31.97 ± 0.41 [ab] (28)	8.88 ± 0.11 [ab] (29)	16.17 ± 0.33 [b] (28)	7.42 ± 0.11 [c] (29)	54.12 ± 0.42 [cd] (28)
Very Old	33.18 ± 0.40 [a] (29)	8.59 ± 0.11 [b] (30)	18.44 ± 0.32 [a] (29)	7.15 ± 0.11 [c] (30)	53.57 ± 0.42 [d] (29)
Interactions					
Ross * Young	27.10 ± 0.60 [a] (13)	8.81 ± 0.15 [a] (15)	14.68 ± 0.48 [a] (13)	8.76 ± 0.16 [a] (15)	60.28 ± 0.62 [a] (13)
Ross * Peak	29.64 ± 0.72 [a] (9)	8.59 ± 0.15 [a] (15)	15.69 ± 0.58 [a] (9)	8.47 ± 0.16 [a] (15)	56.96 ± 0.79 [a] (9)
Ross * Post peak	30.97 ± 0.63 [a] (12)	8.99 ± 0.15 [a] (15)	15.41 ± 0.50 [a] (12)	8.93 ± 0.16 [a] (15)	55.38 ± 0.65 [a] (12)
Ross * Mature	31.97 ± 0.60 [a] (14)	9.13 ± 0.15 [a] (14)	18.11 ± 0.50 [a] (14)	9.07 ± 0.17 [a] (14)	54.63 ± 0.62 [a] (14)
Ross * Old	31.79 ± 0.58 [a] (14)	8.83 ± 0.15 [a] (15)	16.32 ± 0.47 [a] (14)	7.37 ± 0.16 [a] (15)	54.08 ± 0.60 [a] (14)
Ross * Very Old	33.22 ± 0.58 [a] (14)	8.44 ± 0.15 [a] (15)	17.94 ± 0.47 [a] (14)	7.01 ± 0.16 [a] (15)	53.27 ± 0.60 [a] 14)
Cobb * Young	24.51 ± 0.58 [a] (14)	8.53 ± 0.15 [a] (15)	13.62 ± 0.47 [a] (14)	8.49 ± 0.16 [a] (15)	63.13 ± 0.60 [a] (14)
Cobb * Peak	30.24 ± 0.58 [a] (14)	8.82 ± 0.15 [a] (15)	14.97 ± 0.47 [a] (14)	8.45 ± 0.16 [a] (15)	56.03 ± 0.60 [a] (14)
Cobb * Post peak	30.89 ± 0.58 [a] (14)	9.07 ± 0.15 [a] (15)	15.57 ± 0.47 [a] (14)	9.02 ± 0.16 [a] (15)	55.36 ± 0.60 [a] 14)
Cobb * Mature	32.33 ± 0.60 [a] (13)	8.91 ± 0.15 [a] (14)	18.18 ± 0.48 [a] (13)	8.85 ± 0.17 [a] (14)	54.55 ± 0.62 [a] 13)
Cobb * Old	32.13 ± 0.58 [a] (14)	8.93 ± 0.15 [a] (14)	16.02 ± 0.47 [a] (14)	7.46 ± 0.17 [a] (14)	54.16 ± 0.60 [a] 14)
Cobb * Very Old	33.14 ± 0.56 [a] (15)	8.53 ± 0.15 [a] (15)	18.93 ± 0.45 [a] (15)	7.29 ± 0.16 [a] (15)	53.86 ± 0.58 [a] (15)

[a - d] columnar means with different superscripts differ significantly ($P \leq 0.05$).
[1] % wet yolk = (wet yolk weight/initial egg weight) * 100.
[2] % wet shell = (wet shell weight/initial egg weight) * 10.,
[3] % dry yolk = (dry yolk weight/initial egg weight) * 100.
[4] % dry shell = (dry shell weight/Initial egg weight) * 100.
[5] % wet albumen = (final egg weight - (wet yolk + wet shell)) * 100.
[6] (n) = sample size.

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CHAPTER 3: *Experiment 2: To Determine if Broiler Genetic Strain and Parent Flock Age Influence Embryonic Metabolism in Chicken Embryos.*

INTRODUCTION

The performance of the modern broiler represents one of the most marked increases in livestock productivity achieved by selective breeding over the last 50 years. In association with the continuing selection for improved production performance, there have been changes in carcass composition and body conformation, growth, metabolism, digestion, endocrine and immune function, brain function, and behavior (Ellendorff et al., 1995). To date little research has been conducted to characterize possible changes in embryonic development.

Research has shown that breeding and selection has transferred some metabolic imbalances to the developing chick embryo compared to their ancestors (Julian, 1998). Genetic selection for higher meat yielding birds occurred at the same time as developments in large scale incubation; correspondently embryonic mortality has increased (Romanoff, 1949). Commercial hatcheries have noted that broiler strains selected for growth have a higher output of metabolic heat especially starting around 16 d of incubation. To increase hatchability, current commercial incubators need design specifications that will help remove the associated heat resulting from incubating eggs of high meat-yielding broiler breeders. Without this the high embryonic temperature either in the setter or the hatcher will continue to result in brain lesions, shortened hatch time, decreased hatchability, unhealed navels and decreased growth and feed efficiency after hatching (Hulet and Meijerhof, 2001).

To discuss embryonic metabolism, it is important to know the type and amount of fuel (carbohydrate, protein, and fat) used during metabolism. During the latter part of incubation the heat resulting from embryonic metabolism of these food substrates help to maintain multistage incubator temperature. The temperature of the late incubation embryo can be higher than that of the surrounding atmosphere, and the amount of heat produced is roughly proportional to the mass of active tissues. Therefore, when the embryo has attained significant size, the heat radiating from its surfaces modifies temperature conditions in the whole egg (Murray, 1926). In embryos of modern broilers an elevated shell temperature has been linked with higher metabolic activity (Lourens et al., 2005).

Tona et al. (2004) measured physiological indicators such as T_3, T_4 and corticosterone levels in the blood plasma of three strains described as (standard heavy (S) line, an experimental (E) line and a labeled-type (L) line) with the last two carrying dwarf genes. These hormones, when released into the blood stream control metabolism (conversion of oxygen and calories to energy). Every cell in the body depends upon these thyroid hormones for regulation of their metabolism. They can signal low sugar levels and influence the release of glucagon to convert glycogen to glucose. The authors also measured the partial pressure of O_2 (pO_2) and CO_2 (pCO_2) in the egg air cells, and O_2 consumption and CO_2 production of eggs based on paramagnetic and infrared measures respectively. The results showed that the levels of the metabolic indicators (T_3, T_4, and corticosterone) were consistently higher in S-line embryos especially during the late periods of incubation, an indication of higher metabolism during the period of pipping and hatching. They also observed that pCO_2 and heat production of

the S-line was higher than the E-line which was also higher than the L-line. These results are consistent with data from an earlier study where it was observed that embryos of modern high yielding strains produced more heat during incubation than more classical strains (Hulet and Meijerhof, 2001).

Research has also shown that embryonic metabolism increases with increasing flock age (O'Dea et al., 2004). Tullett and Noble (1989) noted that the transfer of lipids from the yolk to the embryo is much reduced in eggs from young parent stock. During development, the embryo uses mainly protein from albumen and fat from yolk for building body tissues. The energy needed for metabolism is obtained by oxidizing (burning) the yolk fat through the ß-oxidation pathway. During this process, the embryo consumes O_2 and produces water, CO_2 and heat. Towards the end of the 21 d incubation period (around 18-19 d) embryonic O_2 demand far exceeds the amount that can be supplied by the shell through simple diffusion to satisfy the metabolic demands for growth and tissue maintenance. Embryos from strains genetically selected for growth may especially be at risk during this incubation period.

A typical value of pre-internal pipping (~18 d incubation) (PreIp) P_{O_2} and P_{CO_2} for all chicken eggs is approximately 104 mmHg and 41 mmHg, respectively (Rahn et al., 1974). However, calculations of O_2 diffusive shell conductance based on measured water vapor eggshell conductance (Paganelli, 1980), and the rate of O_2 consumption during the PreIp stage suggest that PreIp P_{O_2} and P_{CO_2} are allometrically related to egg mass. Thus small and large eggs have low and high PreIp P_{O_2} respectively. During PreIp, when P_{O_2} is higher, egg content temperature is about 1-2.5°C higher than the incubator temperature due to metabolic heat production. As a

result, for a given incubation temperature, egg temperature and heat output may increase with egg mass or flock age related weight increases and cause harm to the embryo (Meir and Ar, 1990).

Research shows that every litre of O_2 consumed by a chicken embryo is equivalent to the production of 4.69 kcal of heat (Vleck et al., 1980a). Typically, O_2 consumption of a chicken egg just before pipping is 570 mL/d (Vleck and Vleck, 1987), equivalent to heat production of 2.67kcal/d or 130 mW. However, this data was obtained from old chicken strains. Segura et al. (2006) observed 3 to 480 mL/d of CO_2 production in chicken embryos at 0 and 18 d of incubation, respectively. For the same days of incubation the calculated heat production values ranged from 1 to 137 mW. The genetic strain of chicken that was investigated was not indicated.

In critical periods of high embryonic heat output any extra heat supplied by the incubating machine or lack of excess heat removal can be detrimental to the embryo because of overheating. To measure embryonic heat production Romijin and Lokhorst (1960) showed that it can be estimated directly by measuring O_2 consumption and CO_2 production. Vleck et al. (1980), comparing the direct methods discussed by some authors (Bohr and Hasselbalch, 1903; Romijin and Lokhorst, 1960), concluded that the most common and easiest method to measure heat production will be to indirectly measure CO_2 (Bohr and Hasselbalch, 1900). Since embryo metabolism is almost exclusively fueled by lipids, with RQ of 0.71 (Rahn et al., 1974), the O_2 consumption and thus the embryo's metabolism can be estimated.

Based on recent considerations about the limitations of the direct methods of measuring heat production (Rahn et al., 1979), Segura et al. (2006) developed a new

metabolic calorimeter system to indirectly measure heat production of domestic avian embryos during incubation. The design of the system is such that it monitors CO_2 and H_2O vapor output and using an RQ of 0.84, the O_2 consumption of the embryo can be estimated. The limitation to this equipment was that O_2 consumption was not directly measured therefore only estimations of O_2 usage were obtained.

Recent reports from hatcheries in Alberta producing chicks with the Cobb 500 strain have indicated that this genetic strain produces more heat than other strains especially between 16 and 18 d of incubation. In addition it has been observed and reported that a egg size increases due to flock aging, embryonic heat production also increases. In commercial hatcheries it has become difficult in modern multistage incubators to manage the eggs for maximum hatchability. As a result of genetic selection for high meat yielding broilers the embryos may also grow fast. This higher metabolism may increase the demand for O_2. in addition the higher embryonic metabolism will increase metabolic heat production especially during latter part of incubation. This could create situations in which the embryo is under heat stress coupled with the stress of the hatching process. These embryos may have a reduced survival advantage

Thus the primary purpose of the present study was to examine the effect of broiler genetic strain and parent flock age on direct O_2 consumption, CO_2 production, and calculated embryonic heat production (metabolism) throughout the entire incubation period. Embryos of Cobb 500 strain were compared to Ross 308 to determine if the higher heat production of the Cobb 500 observed in commercial hatcheries can be validated. The research also studied the effects of genetics and parent

flock age on hatched chick carcass characteristics as measures of chick quality. The hypothesis tested was that embryonic metabolism will be higher in Cobb 500 than Ross 308 strains and that metabolism will also increase as broiler parent flock age increases. Because of the limitations of equipment and experimental designs stated above, a secondary objective of this research was to develop equipments to directly measure O_2 consumption and increase the accuracy of calculated embryonic heat production.

MATERIALS AND METHODS

Experimental Materials

Six trials were conducted in this experiment using two broiler breeder genetic strains (Ross 308 and Cobb 500) at the same broiler flock ages. Experimental eggs were classified according to parent flock age (Young (Y), Peak (P), Post peak (PP), Mature (M), Old (O), and Very Old (VO)) corresponding to 29, 34 (Ross) or 36 (Cobb), 40, 45, 55, and 59 wk old flocks, respectively. Breeder flocks were grouped into six trials as in experiment 1 and egg size remained the same in all trials.The same flocks were used for all the trials excluding the P flocks.

Experimental Design and Incubation. Eggs were incubated using a Jamesway AVN[1] single stage incubator set at 37^0C and approximately 56% relative humidity. The incubator contained 24 identical one litre airtight metabolic chambers.

[1]Jamesways AVN Incubator Company Inc. Cambridge, ON, Canada, N1R 7L3.

In each of the six trials corresponding to the six broiler parent ages, ten eggs from each of Ross 308 and Cobb 500 strains were randomly placed in one of 20 of the 24 metabolic chambers. Five extra eggs per strain were placed in another small Jamesway AVN incubator as spare eggs. After 7 d of incubation, all eggs in the metabolic chambers were candled and the O_2 consumption and CO_2 production values reviewed. Any eggs that were suspected of having nonviable embryos were removed and replaced with an egg from the other incubator.

There were three rows, and two columns of metal racks in the incubator and each row within a column held four metabolic chambers (Figure 3). Within each metabolic chamber was placed a modified plastic incubator egg flat, cut to fit the bottom of the chamber. The egg flat allowed the egg to stay in position while the metal frame work holding the metabolic chambers turned 45^0 to the left and right during incubation

At 18 d of incubation the plastic egg flats were removed to allow the eggs to lie on their sides for the remaining 3 d of incubation as would normally occur in a hatchr.. Beginning at 19 d of incubation, the metabolic chambers were checked every 6 h for hatched chicks. Chicks with dry down were removed and weighed. The length of chick defined as the distance between the tip of the beak and the longest toe of the chick, and the shank length were taken. Each chick was then euthanized via cervical dislocation and the yolk sac removed. After the weight of the chick carcass and yolk sac were taken, the two samples were kept in a freezer until all eggs had hatched. The carcass and yolk sac were then thawed and placed in a drying oven[2] at 65^0C for 4 d to obtain their

[2] Despatch V Series Model VRC2-26-1E, Minneapolis, MN. 55044.

dry weights. After 22 d of incubation all unhatched eggs were broken open to determine the approximate day of embryonic death.

Embryonic Oxygen Consumption and Carbon Dioxide Production

Twenty of the 24 metabolic chambers were used to monitor embryonic O_2 consumption, CO_2 output and eggshell temperature. Three of the four empty metabolic chambers, one on each row were connected together to form a single unit to monitor ambient CO_2. The entire inside of the incubator was used to monitor ambient O_2. However the three empty metabolic chambers were used as a check on the accuracy of the reference O_2 reading.

Description of Metabolism Equipment. Each of the 24 metabolic chambers was connected to a three way electric solenoid valve[3] (located outside the incubator) which controlled air flow through the metabolic chambers. These solenoid valves were operated by a relay or gas channel switcher located in a valve control box housed on top of the incubator. When an electric solenoid valve was on, the air from that metabolic chamber was directed for analysis into two different vacuum manifolds, first; the CO_2/H_2O analyzer[4] and then the differential O_2 analyzer (**DOX**)[5]. The sampling of air from the chambers was pulled into the CO_2/H_2O analyzer every 5 min using a sample pump[6] set daily at a constant flow rate of 300mL/min. In this way the air flow through one metabolic chamber could be monitored, while the other 23 metabolic chambers are closed by their solenoid valves.

[3] ASCO Valve Canada, Brantford, ON, Canada.
[4] Model LI- 6262, LI-COR Inc., Lincoln, NE.
[5, 6] Qubit Systems Inc. 4000 Bath Road, 2nd Floor, Kingston, ON, Canada, K7M 4Y4.

A second pump set up for the DOX reference inlet draws air directly from the incubator. Airflow through the metabolic chambers was adjusted daily by flow meters connected to the solenoid valves. During the first 7 d of incubation (in last two trials) the flow rate was set at 150 ml. For the rest of incubation the air flow was set at 300mL/min. These flow meters were calibrated at the beginning of each experiment by a standardized external flow meter (Dry Cal)[7]. While the CO_2/H_2O analyzer operated from absolute mode in recording CO_2 data, the DOX operated in a differential mode to balance two O_2 partial pressures between its two inlet ports; sample and reference inlet cells to give embryonic O_2 consumption in parts per million (ppm). (In periods when the software readings were altered by calibration error, the two O_2 partial pressure were used in a difference mode to arrive the differential O2 consumption). This works because while the sample cell recorded O_2 consumption (as pressure depression) from air drawn from a metabolic chamber, the reference cell (assisted by the reference pump) draws air from the incubator to give the ambient O_2 partial pressure.

Only 25 psi/25mL/min (see appendix) of air pulled from the CO_2/H_2O analyzer entered into the DOX. Air through the DOX was operated by two DOX controller pumps[8]. One pump was used to obtain air from the air sample going thorough the CO_2/H_2O analyzer. The other pump pulled a sample of air coming directly from the incubator. The pumps were set to pull only 25 ml/min or psi of air through each of the DOX inlets ports. In this way a zero differential pressure between the sample and reference cells of the DOX can be attained to prevent damage to the DOX active cells.

[7, 8] Qubit Systems Inc. 4000 Bath Road, 2nd Floor, Kingston, ON, Canada, K7M 4Y4.

This entire metabolism set up, where changes in CO_2 and O_2 concentrations due to respiration are monitored continuously and not in separate pathways, is called the open flow mode. The entire metabolism equipment as arranged is described in the appendix. Daily calibration of the CO_2/H_2O analyzer and the DOX were done with certified N gas and certified span gas with the following composition (CO_2 = 3038pmm, O_2 = 21.02%) and balanced with N.

DOX Initial Calibration. Measurements of absolute CO_2 and differential O_2 are greatly influenced by fluctuations in atmosphere pressure, air concentration and temperature. It was thus important to calibrate all the gas exchange systems to eliminate the source of these pulsations. At the start of every trial both CO_2/H_2O and differential O_2 analyzers were calibrated to standard settings. The CO_2/H_2O analyzer was calibrated with procedures used by O'Dea et al. (2004). The DOX sample and reference O_2, differential O_2, atmospheric pressure, and differential pressure zero sensors were also calibrated following the principles and procedures provided in the (Qubit Systems Operation Inc., 2005).

To test the integrity of the measured O_2 and CO_2 gases by the O_2 and CO_2 analyzers, propane gas, which has a standard RQ of 0.6, was burnt through the two vacuum manifolds. The O_2 consumed from room air to oxidize 1 mole of propane gas into CO_2, H_2O and energy (McDermott and Feddes, 1991) resulted in RQ of 0.58 and 0.62 (average = 0.6).

Eggshell Temperature. Custom made temperature probes[9] were mounted inside each metabolic chamber. The probes were held in position by sliding the probe though a piece of foam attached to the incubator flat. The positioning of the piece of foam meant that the probe came into contact with the eggshell. The temperature probes in the three empty chambers were connected together and monitored the ambient temperature of the chambers while another temperature probe fixed to the inside of the incubator monitored the ambient temperature within the whole incubator. Readings from the temperature probes were synchronized with O_2 and CO_2 readings, so that eggshell temperature in each chamber was concurrently taken when O_2 and CO_2 were obtained every 5 min. Eggshell temperature was monitored during the first 18 d of incubation.

Software and Data Logging

Both CO_2/H_2O and differential O_2 analyzers were electrically connected to a data acquisition junction box or digital input/output serial port interface. This box receives cables from all instruments and assigns them to specific channels for communication with a personal computer.

A National Instrument data acquisition card[10] and Lab VIEW software[11] running in Microsoft Word were installed onto the computer to provide the requisite number of analog and digital channels for data acquisition and downloading. The relay of all the electric solenoid valves, the CO_2/H_2O analyzer and DOX were operated by the software. The software controlled the switching of solenoid valves to enable monitoring different metabolic chambers every 5 min.

[9] NTC Thermistor, 235-1059, Digi-Key, Thief River Falls, MN.
[10] National Instruments Inc. 11500 N MoPac, Expwy, Austin, TX 78759-3504.
[11] Qubit Systems Inc. 4000 Bath Road, 2nd Floor, Kingston, ON, Canada, K7M 4Y4.

The software was use to arrange the sampling of chambers into a 48 metabolic chamber sequence such that the reference chamber is monitored before any chamber containing an egg is monitored. This resulted in six readings per day per each egg monitored. During the last 10 s of the 5 min sampling period, average embryonic O_2 consumption and CO_2 output were logged into a Microsoft Excel data sheet in the computer as differential O_2 and CO_2 in ppm. These values were the difference between O_2 or CO_2 concentration in a chamber containing an egg and the incubator, and a chamber containing an egg and the reference chambers, respectively. Eggshell temperature was read through a RS-485 PC interface[12] to a different data acquisition[13] sheet on another computer. For the last three trials the RS-485 was built into the Lab VIEW software so that eggshell temperatures, CO_2 and O_2 readings were logged into the same data sheet on the same computer.

Embryonic Heat Production

Embryonic heat output was calculated as a function of calculated O_2 and CO_2 exchange rate in L/s. The following equations were used based on the sample airflow rate of 0.15 (first 7 d in last two trials) or 0.3L/min or (0.0025 or 0.005L/s, respectively) and embryonic heat production estimated by the method of Kleiber (1987) as described by O'Dea et al. (2004). In addition the respiratory quotient was calculated as the average amount of O_2 consumed divided by the average amount of CO_2 produced.

[12] 485 DRJ, B & B Electronics Manufacturing Company, Ottawa, IL.
[13] Remote data acquisition PC interface, 485 SDA12, B & B Electronics Manufacturing Company.

O_2 or CO_2 exchange rate of embryo (L/s)

$$= \frac{O_2 \text{ consumption or } CO_2 \text{ production (ppm) x sample airflow rate (L/s)}}{10^6}$$

Heat production (mW) = [3.871 x O_2 consumption (L/d) + 1.194 x CO_2 production (L/d)] x 1 d / 24h x 1 h / 3600s x 1000cal / 1 kcal x 4.187 J / cal x 1000 mW/W.

Statistical Analysis

Oxygen consumption, CO_2 production and eggshell temperatures, and calculated heat production were analyzed as a single dependent variable in repeated measures data (six reading taken per day), using the Generalized Linear Model Procedure of SAS® (SAS, 2005) and means separated as lsmeans. The repeated measures single dependent variable was evoked as the number of missing data was very high, such that deleting them could have lead to loss of a whole day's data. Where significant differences were observed between lsmeans, they were separated by the pdiff procedure SAS® at $P < 0.05$. Time taken to externally pip and hatch, wet and dry chick carcass and wet and dry yolk sac weights were analyzed as 2 X 6 factorial design of the Generalized Linear Model of SAS® (SAS, 2005) and means separated as lsmeans. Where significant differences were observed lsmeans were again separated using the pdiff procedure of SAS®. Significance was based on $P < 0.05$. Unless otherwise specified, values are reported as the lsmeans ± standard error of the mean.

RESULTS AND DISCUSSION

Egg Weight, External Pipping and Hatching Time

Effect of Breeder Strain. Average egg weight at setting was significantly higher in the Ross strain than the Cobb strain (Table 3). However, at transfer, the weight differences between the two strains were not significantly different. The time taken to pip was significantly higher in Cobb eggs than Ross eggs by almost 5.5 h, but the time taken to hatch was not different between the two strains. This may be an indication of different growth strategies between the two strains. In addition the early pipping time observed in the Ross embryos implies that they started pulmonary respiration earlier than the Cobb embryos and this may enhance their survival. It is also possible that the Ross strain internally pipped earlier. If this was the case they would take longer to through the stpes of hatching I which O2 in sufficient amount to sustain the embryo is not available. This might give an advantage to the Cobb strains in terms embryo survival and hatchability. Future research should aim to determine both internal and external pipping times to answer this question.

Effect of Breeder Age. Table 3 shows that average egg weight at setting was significantly lower in the young (Y) (29 wk) breeder flocks compared to all other flock ages. At the time of transfer, there were no differences in egg weight between the flock ages. The time taken to externally pip through the eggshell was longest in very old (VO) and mature (M) flocks compared to all other flock ages. Peak (P) and post peak (PP) flocks took longer to pip than embryos from young (Y) and old (O) flocks. Apart from the O flocks, as flock age increased, the pipping time increased. These results are very

interesting especially in view of the fact that the egg weights at transfer for all the flock ages were equal. This provides strong evidence that flock age influence external pipping time independent of egg weight. These results contradict previous research showing that older flocks will usually pip earlier than those from younger flocks (Peebles and Brake, 1987).

Peebles and Brake (1987) noted that the O_2 demand of embryos prior to pipping is greater in larger eggs than it is in smaller ones due to embryo size. Therefore the demand for O_2 will trigger pipping earlier in older flocks than eggs from younger flocks. However one factor than could possibly influence the early pipping in the younger flocks is accumulation of CO_2 in the egg. The thicker eggshell and the narrow eggshell pores in younger flocks may cause a build up CO_2 and thus stimulate the embryo to pip.

Chicks from VO flocks took the longest time to hatch followed by M, PP and Y flocks. The PP and Y flocks were not different from P flocks which did hatch earlier compared to M and VO flocks. Embryos from O flock hatched the earliest of all flock ages. The general pattern was that as flocks aged the time to hatching increased. The exception in this experiment was the O flocks which hatched the earliest. An explanation for this cannot be provided

In a recent study using Ross 308 X Ross broiler crosses, Joseph and Moran (2005) found that prime flocks (41 wk old) produced more early hatched chicks compared to young parent flocks (32 wk old). This disagrees with the current research results where 34 or 36 wk (P) chicks hatched at the same time as 40 wk flocks (PP) but earlier than 45 wk (M) flocks. The initiation of pulmonary respiration as soon as

embryos internally and externally pip may stimulate faster hatching due to embryos coming out of the hypoxia stage quicker. It is therefore logical to assume that the chicks that pip early would hatch early excluding the VO flock. This was not the case in the current experiment.

Van de Ven (2005) observed that from the moment of oviposition, eggs from older flocks (>32 weeks) already contain embryos in a more advanced stage of development that cause the embryos to develop at a higher rate than embryos in eggs from younger flocks. Consequently, embryos in eggs from the latter group require longer incubation periods. The author reported further that some studies even state a decrease in incubation time of up to 10 h between flocks at 28 and 32 wk of maternal age. In two separate experiments, Suarez et al. (1997) reported that incubation time decreased from 499 h (when breeders were 29 wk) to 495 h at 47 wk and 511 h at 29 wk to 495 h at 41 wk. All these findings contradict with the current research. On the contrary, Shanawany (1984) noted that eggs from younger parents hatch earlier than older flocks. Egg size may play more of a role than parent flock age with respect to hatching time.

Effect of Breeder Strain and Breeder Age Interaction. From Table 3, both egg weight at setting and weight at transfer were not affected by breeder strain and breeder flock age interactions. However there were significant effect of the interactions on pipping and hatching times. Looking at the Ross strain there appears to be no clear cut trend regarding the pipping times. For the hatching times, however in general hatching time increased as flock age increased in the Ross strain. The exception was the O flocks

which pipped and hatched very early compared to the other flock ages. A similar trend was noted in the Cobb flocks; excluding the O flock, as the flock aged pipping time increased. All these differences occurred even though egg sizes were the same. This indicates that the interactions of the two factors have effect on external pipping time and hatching time.

Chick Weight, Chick Length and Shank Length

Effect of Breeder Strain. Results from Table 4 show that eggs from the R strain hatched heavier chicks than C strain. This could be a reflection of egg weight differences (Table 3). A study by Shanawany (1984) showed that there is a positive correlation between egg weight and chick weight and that bigger eggs will hatch bigger chicks. Chick length and shank length were not affected by genetic strain.

Effect of Breeder Age. Chicks from VO parents had significantly heavier chicks compared to all other flock ages which were not different from each other. However, in the current research, egg set weights and egg transfer weights were not significantly heavier in the VO flocks than the other flock ages. The results thus indicate that chick weight is heavier in old flocks independent of egg weight. Chicks from VO flocks were longer than P and PP flock chicks, but the three age groups were not different from chicks from Y, M or O flock. This shows that chick length is not a good indicator of chick weight. There is a general concept that longer chicks will have longer shank lengths but this was also not the case. Chicks from P flocks which were one of the two flock ages that were the shortest had the longest shank length followed by PP and O

flocks. Chicks from M flocks had the shortest shank. The data show that shank length is not a good indicator of chick length.

Effect of Breeder Strain and Breeder Age Interaction. The Ross chicks from the VO flocks were the heaviest. The weights of the chicks at hatching were not as a result of heavier set eggs or transfer weights, and did not show a clear pattern related to hatching time (Table 4). The interaction of genetic strain and parent flock age (Table 4) had no effect on chick or shank lengths.

Percentage Chick Carcass and Yolk sac Weights

Effect of Breeder Strain. The results indicate that genetic strain had no significant effect on percentage wet carcass, wet yolk sac, dry carcass and dry yolk sac (Table 5). It is possible that the result was because egg weights were the same between the two strains investigated.

Effect of Breeder Age. Table 5 shows the results of carcass analysis after hatching. The percentage yolk free body mass (called wet chick carcass) was significantly higher in Y and P flocks compared to O flocks but there was no difference from any other flock ages. However, percentage dry carcass weight in PP flocks were greater than any other flock ages, which did not differ from one another. For yolk sac, the results show a general trend of increasing percentage wet and dry yolk sac as flock age increased (Table 5). Similarly Peebles et al. (2001) found that as broiler flock age increased from 26 to 31 wks and from 31 to 35 wk, yolk weight also increased. Suarez

et al. (1997) reported higher percentage yolk in 59 wk old flocks compared to 29 wk old flocks. Looking at results described earlier (Table 4), it is likely that the heavier weight of chicks from VO flocks was a direct effect of yolk sac mass.

Effect of Breeder Strain and Breeder Age Interaction. There were no significant effect of breeder strain and breeder flock age interaction on percentage wet carcass, dry carcass and wet and dry yolk sac contents (Table 5). The fact that egg weight was kept the same between the strains and flock ages contributed to this.

Embryonic Oxygen Consumption and Carbon Dioxide production

Effect of Breeder Strain. Both average and total embryonic O_2 consumption and CO_2 production over the entire incubation period were not significantly different between Ross and Cobb embryos (Table 6). However there were daily differences in average O_2 uptake 1, 7, 16, 17, 19, 20 d of incubation between the two strains (Table 7 and Figure 4). While the Cobb strains consumed more O_2 on d 1, the Ross strain had higher consumption at d 7. During the 16 and 17 d of incubation the Ross embryos consumed more O_2, but Cobb embryos consumed more O_2 on the 19 and 20 d of incubation.

Daily embryonic CO_2 output differed between the two strains during the very early stages of incubation 1- 4 d and then in the latter then in the later periods of incubation 16-20 d (Table 7 and Figure 5). During the first 4 d of incubation the Cobb strain produced more CO_2 than the Ross strain. In the later period of incubation the Ross strain produced more CO_2 than the Cobb embryos on 16, 17, 18, and 19 d of incubation.

Though O_2 consumption differed between the genetic strains on only 1 and 7 d of incubation, these results together with the CO_2 data show a higher metabolic rate in the Cobb strain than the Ross strain during the first wk of incubation. This period of incubation is a critical time during which organ formation is occurring. The O_2 requirement may also be higher due to a larger embryonic body size in the Cobb strains which are genetically selected for growth. Future research should include daily embryonic weights to establish if embryo weight is the reason for higher metabolism in the Cobb 500 during the first wk of incubation.

Around the 16 and 17 d of incubation, there is massive organ and tissue growth and organs such as the heart and brain require more O_2 to coordinate the incorporation of metabolites such as proteins and fat into body building (Brake et al., 1997). The beginning of 18 d of incubation has been identified as the start of the plateau stage of O_2 consumption (Wineland et al., 2006) when O_2 demand exceeds O_2 that can be supplied by simple diffusion through the shell. The data show that the Ross embryos had higher O_2 consumption and CO_2 production than Cobb embryos at 16 and 17 d. This was unexpected as it was hypothesized that the Cobb 500 strains, selected for growth, would have higher O_2 consumption, CO_2 production and heat production during the final days of incubation. This data provides evidence that when egg weight is kept the same, Ross embryos have a higher metabolism during the days prior to internal pipping. However during the time of internal (19 d) and external pipping (20 d) the Cobb embryos consumed more O_2. The Cobb embryos also and produced more CO_2 on 20 d. This shows that the Cobb embryos require more O_2 during the time period when the embryo is exiting the shell.

Effect of Breeder Age. The results from Table 6 illustrate that embryos from VO flocks were consuming more O_2 than embryos from P, PP and M flocks. The O_2 consumption of VO embryos did not differ from embryos of Y and O flocks. This may be indicative of the fact that the VO flock had the highest chick weights at hatching. For future studies it would be valuable to abtain daily embryonic weights for each day of incubation to establish if consumption parallels embryo weight.

Except on d 8 of incubation there were significant differences in daily O_2 consumption among parent flock ages (Table 8 and Figure 6). Embryos from O and VO flocks consistently consumed more O_2 during most of the early and middle periods of incubation (Table 8 and Figure 7) and during 18 to 21 d (Table 8 and Figure 8). Also, there were significant differences between flock ages in embryonic CO_2 production for all days of incubation, excluding 7 and 9 d (Table 9 and Figure 9) For the first 14 d of incubation the PP flocks generally produced more CO_2 than the Y and P flocks (Figure 10). From 15 d to the end of incubation embryos from M parent flocks produced more CO_2 compared to all other flock ages (Figure 11). Collectively these results support the findings of O'Dea et al. (2004) who showed that embryos from older (38 wk) flocks had higher CO_2 production than embryos from younger parent flocks (33 wk).

Effect of Breeder Strain and Breeder Age Interaction. There was no significant effect of the interaction between genetic strain and parent flock age on either average or total O_2 consumption or CO_2 production for the entire incubation period (Table 6).

Eggshell Temperature and Embryonic Heat production

Effect of Breeder Strain. Genetic strain did not significantly influence average eggshell temperature, average embryonic heat production, and total embryonic heat production over the 21 d incubation period (Table 10). However, daily embryonic heat production differences did exist between the two strains on 4, 7, 16, 17, 18 and 19 d of incubation (Table 11; Figure 12). The Cobb strain had higher heat production on 4 and 19 d of incubation, while the embryos from the Ross strain produced more heat on d 7, 16, 17 and 18 of incubation. The commercial observations that Cobb strains produce a lot of heat during latter periods of incubation was validated by the current research showing higher heat production on 19 d of incubation.

This period of excess heat production during the time when rigorous hatching activity is occurring could be making the Cobb embryos more vulnerable to. Tona et al. (2004) explained that during the final stage of incubation heavier broiler strains produced more heat than experimental and labeled-type lines. Generally, it was expected that heat production of the higher breast meat yielding Cobb 500 would be much greater than the Ross 308 throughout incubation. Te fact that there were times during incubation when each of the two strains had higher heat production indicates that there are different metabolic patterns for each strain.

Effect of Breeder Age. The results from Table 10 show that embryos from Y parent flocks had lower average eggshell temperatures compared to embryos from all other flock ages, which did not differ from one another. It is expected that as breeder flock age increased embryonic mass would increases and more embryonic heat would

be produced. This was not the case, likely due to the fact that egg weights were kept the same as the flocks aged. Embryonic heat production in P and PP flocks were significantly lower than all other flock ages including Y flocks (Table 10). This was unexpected and cannot be explained from the data obtained from this research. O'Dea et al. (2004) calculated that embryonic heat production in embryos from a 38 wk old flock was higher than embryos from a 33 wk old flock. The results from the current research (excluding Y flock) support the finding that embryos of older flocks have higher heat production. Average daily heat production was significantly different among the six age groups throughout incubation (Table 12 and Figures 13, 14 and 15) except on the 8th d of incubation. Embryos from O and VO parents consistently produced more heat during most of the days of incubation compared to embryos from other parent flock ages.

Effect of Breeder Strain and Breeder Age Interaction. Apart from eggshell temperature where there were significant interactions between strain and parent flock age, the interplay of genetic strain and parent flock age did not have significant influence on average or total embryonic heat production.

Summary and Conclusion

In the current research embryonic metabolism differences did not exist between the two modern broiler strains when the entire incubation period was considered. However, daily differences did exist during the early and late periods of incubation. This is an important finding as these are the periods during which most embryonic mortality occurs. Results on the effect of broiler parent flock age show that embryonic

metabolism was highest in embryos from O and VO parent flocks. An examination of daily heat production in all parent flock ages especially support the statement that as breeder flock age increases, embryonic metabolism also increases. The results from this research show that strain and flock age do influence daily embryonic metabolism. This knowledge may assist hatchery managers when deciding which eggs to place together in an incubator. Future research should examine different temperature profiles during incubation in order to optimize embryo survival.

Figure 3: Egg metabolism equipment showing the incubator, metabolic chambers, CO_2/H_2O analyzer, differential O_2, data recording computers and accessory parts

Table 3: Effects of broiler breeder strain and flock age on hatching egg weights and pipping and hatching time

Source	Egg set weight (g)[1]	Transfer weight (g)[2]	Pipping time (h)[3]	Hatching time (h)[4]
Genetic strain				
Ross	64.64 ± 0.04 [a] (60)[5]	58.15 ± 0.24 [a] (60)	474 ± 2.11 [b] (55)	495 ± 0.85 [a] (53)
Cobb	64.50 ± 0.04 [b] (60)	57.62 ± 0.24 [a] (60)	479 ± 2.13 [a] (55)	495 ± 0.84 [a] (55)
Flock age				
Young (29 wk)	64.13 ± 0.07 [b] (20)	58.38 ± 0.41 [a] (20)	456 ± 3.71 [c] (18)	498 ± 1.53 [bc] (16)
Peak (34 or 36 wk)	64.60 ± 0.07 [a] (20)	58.42 ± 0.41 [a] (20)	478 ± 3.94 [b] (16)	492 ± 1.55 [c] (16)
Post peak (40 wk)	64.68 ± 0.07 [a] (20)	57.79 ± 0.41 [a] (20)	481 ± 3.71 [b] (18)	494 ± 1.46 [bc] (18)
Mature (45 wk)	64.71 ± 0.07 [a] (20)	57.09 ± 0.41 [a] (20)	495 ± 3.59 [a] (19)	506 ± 1.41 [b] (19)
Old (55 wk)	64.67 ± 0.07 [a] (20)	57.14 ± 0.41 [a] (20)	456 ± 3.59 [c] (19)	466 ± 1.41 [d] (19)
Very Old (59 wk)	64.63 ± 0.07 [a] (20)	58.46 ± 0.41 [a] (20)	494 ± 3.49 [a] (20)	515 ± 1.37 [a] (20)
Interactions				
Ross * Young	64.56 ± 0.10 [a] (10)	58.81 ± 0.58 [a] (10)	437 ± 4.94 [e] (10)	493 ± 2.17 [d] (8)
Ross * Peak	64.62 ± 0.10 [a] (10)	58.85 ± 0.58 [a] (10)	479 ± 5.21 [bc] (9)	495 ± 2.04 [d] (9)
Ross * Post peak	64.70 ± 0.10 [a] (10)	58.08 ± 0.58 [a] (10)	486 ± 5.52 [b] (8)	494 ± 2.17 [d] (8)
Ross * Mature	64.71 ± 0.10 [a] (10)	56.45 ± 0.58 [a] (10)	498 ± 5.21 [a] (9)	507 ± 2.04 [bc] (9)
Ross * Old	64.64 ± 0.10 [a] (10)	58.18 ± 0.58 [a] (10)	456 ± 5.21 [cd] (9)	467 ± 2.04 [e] (9)
Ross * Very Old	64.61 ± 0.10 [a] (10)	58.51 ± 0.58 [a] (10)	491 ± 4.94 [a] (10)	513 ± 1.94 [ab] (10)
Cobb * Young	63.69 ± 0.10 [a] (10)	57.95 ± 0.58 [a] (10)	475 ± 5.52 [bc] (8)	503 ± 2.17 [c] (8)
Cobb * Peak	64.59 ± 0.10 [a] (10)	57.99 ± 0.58 [a] (10)	477 ± 5.90 [bc] (7)	489 ± 2.32 [d] (7)
Cobb * Post peak	64.66 ± 0.10 [a] (10)	57.51 ± 0.58 [a] (10)	479 ± 4.94 [bc] (10)	495 ± 1.94 [d] (10)
Cobb * Mature	64.71 ± 0.10 [a] (10)	57.73 ± 0.58 [a] (10)	492 ± 4.94 [ab] (10)	505 ± 1.94 [c] (10)
Cobb * Old	64.70 ± 0.10 [a] (10)	56.10 ± 0.58 [a] (10)	456 ± 4.94 [d] (10)	465 ± 1.94 [e] (10)
Cobb * Very Old	64.65 ± 0.10 [a] (10)	58.41 ± 0.58 [a] (10)	498 ± 4.94 [a] (10)	514 ± 1.94 [a] (10)

[a-e] columnar means with different superscripts differ significantly ($P \leq 0.05$).

[1] Egg set weight = Egg weight at time eggs were placed in incubator.

[2] Transfer weight = Egg weight at 18 d of incubation.

[3] Pipping time = Time taken from time of setting eggs in incubator to external pipping (embryo pips through shell).

[4] Hatching time = Time taken from setting eggs in incubator to chick fully hatched and down feathers dry.

[5] (n) = number of experimental eggs.

Table 4: Effect of broiler breeder strain and flock age on hatched chick weight, chick length and shank length.

Source	Chick weight (g)[1]	Chick length (mm)[2]	Chick shank length (mm)[3]
Genetic strain			
Ross	48.22 ± 0.17 ^{a} (53)[4]	181.46 ± 0.59 ^{a} (53)	26.82 ± 0.13 ^{a} (53)
Cobb	47.43 ± 0.17 ^{b} (55)	180.19 ± 0.58 ^{a} (55)	26.68 ± 0.13 ^{a} (55)
Flock age			
Young (29wk)	47.81 ± 0.30 ^{b} (16)	181.13 ± 1.06 ^{ab} (16)	26.59 ± 0.24 ^{bc} (16)
Peak (34 or 36 wk)	47.63 ± 0.31 ^{b} (16)	178.86 ± 1.07 ^{b} (16)	27.86 ± 0.24 ^{a} (16)
Post peak (40 wk)	47.89 ± 0.29 ^{b} (18)	178.56 ± 1.01 ^{b} (18)	26.88 ± 0.23 ^{b} (18)
Mature (45 wk)	47.33 ± 0.28 ^{b} (19)	181.68 ± 0.98 ^{ab} (19)	25.88 ± 0.22 ^{c} (19)
Old (55 wk)	47.04 ± 0.28 ^{b} (19)	180.77 ± 0.98 ^{ab} (19)	26.87 ± 0.22 ^{b} (19)
Very Old (59 wk)	49.25 ± 0.27 ^{a} (20)	183.95 ± 0.95 ^{a} (20)	26.42 ± 0.21 ^{bc} (20)
Interactions			
Ross * Young	48.92 ± 0.43 ^{ab} (8)	181.00 ± 1.50 ^{a} (8)	26.63 ± 0.34 ^{a} (8)
Ross * Peak	48.12 ± 0.40 ^{b} (9)	180.00 ± 1.42 ^{a} (9)	27.92 ± 0.32 ^{a} (9)
Ross * Post peak	48.05 ± 0.43 ^{b} (8)	178.75 ± 1.50 ^{a} (8)	26.80 ± 0.34 ^{a} (8)
Ross * Mature	46.87 ± 0.40 ^{c} (9)	181.67 ± 1.42 ^{a} (9)	25.86 ± 0.32 ^{a} (9)
Ross * Old	47.93 ± 0.40 ^{bc} (9)	183.44 ± 1.42 ^{a} (9)	27.19 ± 0.32 ^{a} (9)
Ross * Very Old	49.41 ± 0.38 ^{a} (10)	183.90 ± 1.34 ^{a} (10)	26.53 ± 0.30 ^{a} (10)
Cobb * Young	46.71 ± 0.43 ^{c} (10)	181.25 ± 1.50 ^{a} (8)	26.55 ± 0.34 ^{a} (8)
Cobb * Peak	47.14 ± 0.46 ^{bc} (7)	177.71 ± 1.61 ^{a} (7)	27.79 ± 0.36 ^{a} (7)
Cobb * Post peak	47.75 ± 0.38 ^{bc} (10)	178.40 ± 1.34 ^{a} (10)	26.96 ± 0.30 ^{a} (10)
Cobb * Mature	47.78 ± 0.38 ^{bc} (10)	181.70 ± 1.34 ^{a} (10)	25.89 ± 0.30 ^{a} (10)
Cobb * Old	46.15 ± 0.38 ^{c} (10)	178.10 ± 1.34 ^{a} (10)	26.56 ± 0.30 ^{a} (10)
Cobb * Very Old	49.02 ± 0.38 ^{ab} (10)	184.00 ± 1.34 ^{a} (10)	26.31 ± 0.30 ^{a} (10)

$^{a-c}$, columnar means with different superscripts differ significantly ($P \leq 0.05$.)

[1] Chick weight = weight of chicks after hatching.

[2] Chick length = the distance between chick beak and tip of the longest toe.

[3] Chick shank length = the distance along the metatarsus.

[4] (n) = number of experimental units.

Table 5: Effect of two broiler breeder strains and six flock ages on percentage chick carcass and yolk sac.

Source	% Wet carcass [1]	% Dry carcass [2]	% Wet yolk sac [3]	% Dry yolk sac [4]
Genetic strain				
Ross	81.23 ± 0.26 [a] (53) [5]	16.15 ± 0.17 [a] (53)	18.01 ± 0.25 [a] (53)	9.87 ± 0.16 [a] (53)
Cobb	81.30 ± 0.26 [a] (55)	16.21 ± 0.17 [a] (55)	17.89 ± 0.25 [a] (55)	9.76 ± 0.16 [a] (55)
Flock age				
Young (29 wk)	81.99 ± 0.47 [a] (16)	15.82 ± 0.36 [b] (16)	16.79 ± 0.45 [b] (16)	8.54 ± 0.28 [c] (16)
Peak (34 or 36 wk)	82.07 ± 0.47 [a] (16)	15.75 ± 0.31 [b] (16)	17.37 ± 0.46 [b] (16)	9.17 ± 0.29 [bc] (16)
Post peak (40 wk)	81.22 ± 0.45 [ab] (18)	18.09 ± 0.29 [a] (18)	18.16 ± 0.43 [ab] (18)	9.96 ± 0.27 [ab] (18)
Mature (45 wk)	81.37 ± 0.43 [ab] (19)	16.18 ± 0.28 [b] (19)	17.88 ± 0.42 [ab] (19)	10.04 ± 0.26 [ab] (19)
Old (55 wk)	79.95 ± 0.43 [b] (19)	15.82 ± 0.28 [b] (19)	19.30 ± 0.42 [a] (19)	10.96 ± 0.26 [a] (19)
Very Old (59 wk)	80.98 ± 0.42 [ab] (20)	15.43 ± 0.27 [b] (20)	18.19 ± 0.41 [ab] (20)	10.24 ± 0.26 [a] (20)
Interactions				
Ross * Young	80.94 ± 0.67 [a] (8)	15.32 ± 0.46 [a] (8)	17.83 ± 0.64 [a] (8)	9.09 ± 0.41 [a] (8)
Ross * Peak	82.18 ± 0.63 [a] (9)	15.57 ± 0.41 [a] (9)	17.24 ± 0.61 [a] (9)	9.08 ± 0.38 [a] (9)
Ross * Post peak	81.38 ± 0.67 [a] (8)	18.50 ± 0.43 [a] (8)	17.95 ± 0.64 [a] (8)	9.95 ± 0.41 [a] (8)
Ross * Mature	82.29 ± 0.68 [a] (9	16.76 ± 0.41 [a] (9)	16.95 ± 0.61 [a] (9)	9.54 ± 0.38 [a] (9)
Ross * Old	79.66 ± 0.63 [a] (9)	15.55 ± 0.41 [a] (9	19.58 ± 0.61 [a] (9	11.21 ± 0.38 [a] (9
Ross * Very Old	80.92 ± 0.59 [a] (10)	15.22 ± 0.38 [a] (10)	18.50 ± 0.57 [a] (10)	10.35 ± 0.36 [a] (10)
Cobb * Young	83.04 ± 0.67 [a] (10)	16.32 ± 0.54 [a] (10)	15.77 ± 0.64 [a] (10)	7.99 ± 0.41 [a] (10)
Cobb * Peak	81.96 ± 0.71 [a] (7)	15.94 ± 0.46 [a] (7)	17.50 ± 0.69 [a] (7)	9.26 ± 0.43 [a] (7)
Cobb * Post peak	81.07 ± 0.59 [a] (10)	17.67 ± 0.38 [a] (10)	18.38 ± 0.57 [a] (10)	9.96 ± 0.36 [a] (10)
Cobb * Mature	80.46 ± 0.59 [a] (10)	15.61 ± 0.38 [a] (10)	18.81 ± 0.57 [a] (10)	10.54 ± 0.36 [a] (10)
Cobb * Old	80.23 ± 0.59 [a] (10)	16.10 ± 0.38 [a] (10)	19.02 ± 0.57 [a] (10)	10.71 ± 0.36 [a] (10)
Cobb * Very Old	81.05 ± 0.59 [a] (10)	15.63 ± 0.38 [a] (10)	17.89 ± 0.57 [a] (10)	10.13 ± 0.36 [a] (10)

[a-c] columnar means with different superscripts differ significantly ($P \leq 0.05$).

[1] % Wet carcass = (wet carcass weight / chick weight) * 100.

[2] % Dry carcass = (dry carcass weight / chick weight) *100.

[3] % Wet yolk sac = wet yolk sac weight / chick weight) * 100.

[4] % Dry yolk sac = (dry yolk sac weight / chick weight) * 100.

[5] (n) = number of experimental units.

Table 6: Effect of two broiler breeder genetic strains and six breeder flock ages on average and total embryonic gas exchange.

Source	Average O_2 consumption (µL/s) [1]	Average CO_2 production (µL/s) [2]	Total O_2 consumption over 21 d (µL) [3]	Total CO_2 Production over 21 d(µL) [4]
Genetic strain				
Ross	4.29 ± 0.06 [a] (6035) [5]	2.67 ± 0.03 [a] (6606)	90.09 [a]	56.07 [a]
Cobb	4.16 ± 0.06 [a] (6028)	2.61 ± 0.03 [a] (6558)	87.36 [a]	55.02 [a]
Flock age				
Young (29 wk)	4.45 ± 0.11 [ab] (6028)	2.57 ± 0.06 [b] (2138)	93.45 [ab]	53.97 [b]
Peak (34 or 36 wk)	3.86 ± 0.12 [c] (1671)	2.66 ± 0.06 [ab] (1940)	81.06 [c]	55.86 [ab]
Post peak (40 wk)	3.85 ± 0.10 [c] (2207)	2.62 ± 0.06 [ab] (2239)	80.85 [c]	55.02 [ab]
Mature (45 wk)	4.20 ± 0.11 [bc] (1964)	2.81 ± 0.06 [a] (2385)	88.20 [bc]	59.01 [a]
Old (55 wk)	4.39 ± 0.10 [ab] (2171)	2.69 ± 0.06 [ab] (2168)	92.19 [a]	56.49 [ab]
Very Old (59 wk)	4.62 ± 0.10 [a] (2152)	2.48 ± 0.06 [b] (2294)	97.02 [a]	52.08 [b]
Interactions				
Ross * Young	4.56 ± 0.15 [a] (1025)	2.62 ± 0.08 [a] (1158)	95.76 [a]	55.02 [a]
Ross * Peak	3.85 ± 0.16 [a] (872)	2.66 ± 0.09 [a] (1018)	80.85 [a]	55.86 [a]
Ross * Post peak	3.79 ± 0.15 [a] (1031)	2.59 ± 0.09 [a] (1048)	79.59 [a]	54.39 [a]
Ross * Mature	4.25 ± 0.15 [a] (989)	2.84 ± 0.08 [a] (1196)	89.25 [a]	59.64 [a]
Ross * Old	4.55 ± 0.15 [a] (1028)	2.78 ± 0.09 [a] (1026)	95.55 [a]	58.38 [a]
Ross * Very Old	4.76 ± 0.15 [a] (1090)	2.53 ± 0.08 [a] (1160)	99.75 [a]	53.13 [a]
Cobb * Young	4.35 ± 0.16 [a] (873)	2.52 ± 0.09 [a] (980)	91.35 [a]	52.92 [a]
Cobb * Peak	3.86 ± 0.17 [a] (799)	2.67 ± 0.09 [a] (922)	81.06 [a]	56.07 [a]
Cobb * Post peak	3.89 ± 0.14 [a] (1176)	2.66 ± 0.08 [a] (1191)	81.69 [a]	55.86 [a]
Cobb * Mature	4.15 ± 0.15 [a] (975)	2.78 ± 0.08 [a] (1189)	87.15 [a]	58.38 [a]
Cobb * Old	4.25 ± 0.14 [a] (1143)	2.59 ± 0.08 [a] (1142)	89.25 [a]	54.39 [a]
Cobb * Very Old	4.49 ± 0.15 [a] (1062)	2.43 ± 0.08 [a] (1134)	94.29 [a]	51.03 [a]

[a-c], columnar means with different superscripts differ significantly ($P \leq 0.05$).

[1] Average O_2 consumption = sum of average daily O_2 consumption / 21d incubation.

[2] Average CO_2 production = sum of average daily CO_2 output / 21 d of incubation.

[3] Total O_2 consumption over 21 d = sum of all average daily O_2 consumption for all 21 d of Incubation.

[4] Total CO_2 production over 21 d = sum of all average daily CO_2 production for all 21 d of Incubation.

[5] (n) = number of experimental readings taken during the 21 d of incubation.

Table 7: Effect of breeder strain on average daily embryonic O2 consumption and CO2 production (μL) over 21 d incubation period.

	O_2 consumption (μL)		CO_2 production (μL)	
Day	Ross 308	Cobb 500	Ross 308	Cobb 500
1	0.04[b]	0.05[a]	0.0[b]	0.06[a]
2	0.06[a]	0.06[a]	0.04[b]	0.05[a]
3	0.07[a]	0.08[a]	0.06[b]	0.07[a]
4	0.13[a]	0.14[a]	0.13[b]	0.14[a]
5	0.25[a]	0.25[a]	0.22[a]	0.23[a]
6	0.45[a]	0.45[a]	0.27[a]	0.27[a]
7	0.46[a]	0.41[b]	0.34[a]	0.32[a]
8	0.66[a]	0.74[a]	0.47[a]	0.46[a]
9	0.92[a]	0.90[a]	0.65[a]	0.66[a]
10	1.37[a]	1.35[a]	0.85[a]	0.84[a]
11	1.96[a]	1.92[a]	1.21[a]	1.19[a]
12	2.78[a]	2.78[a]	1.76[a]	1.80[a]
13	3.91[a]	3.83[a]	2.60[a]	2.62[a]
14	5.77[a]	5.64[a]	3.60[a]	3.54[a]
15	7.35[a]	7.32[a]	4.74[a]	4.7[a]
16	8.84[a]	8.62[b]	5.63[a]	5.53[b]
17	9.45[a]	9.28[b]	6.08[a]	5.95[b]
18	10.11[a]	9.29[a]	6.19[a]	6.01[b]
19	9.21[b]	9.61[a]	6.40[a]	6.23[b]
20	10.68[b]	11.05[a]	7.00[b]	7.19[a]
21	13.96[a]	13.58[a]	9.27[a]	9.34[a]

[a-b] columnar means with different superscripts differ significantly ($P \leq 0.05$).

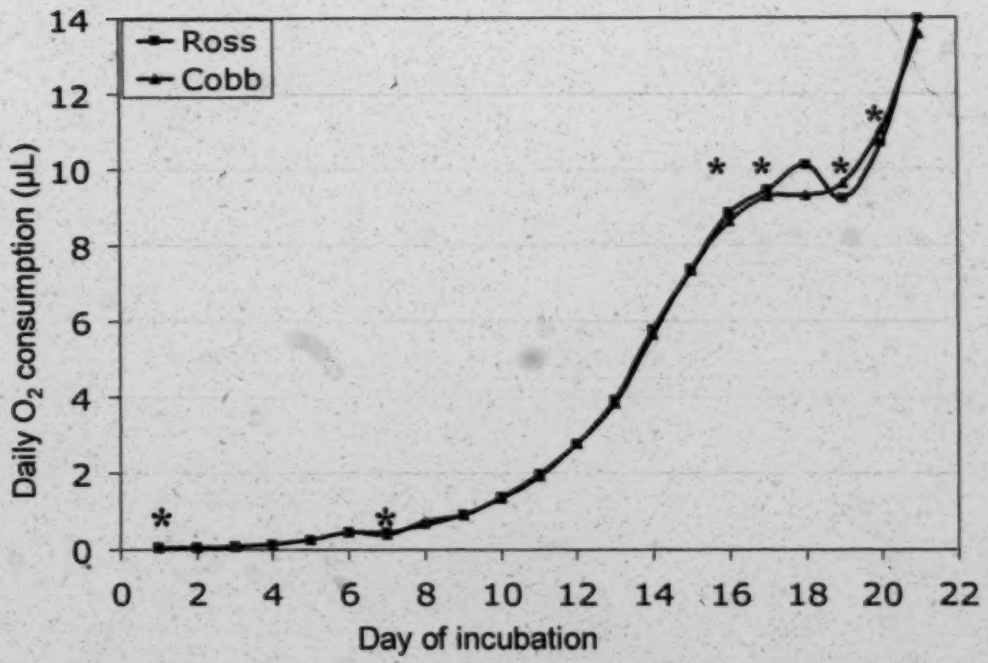

Figure 4: Effect of breeder strain on average daily embryonic O_2 consumption (µL) over 21 d incubation period (* indicates significance difference between the two strains ($P \leq 0.05$)).

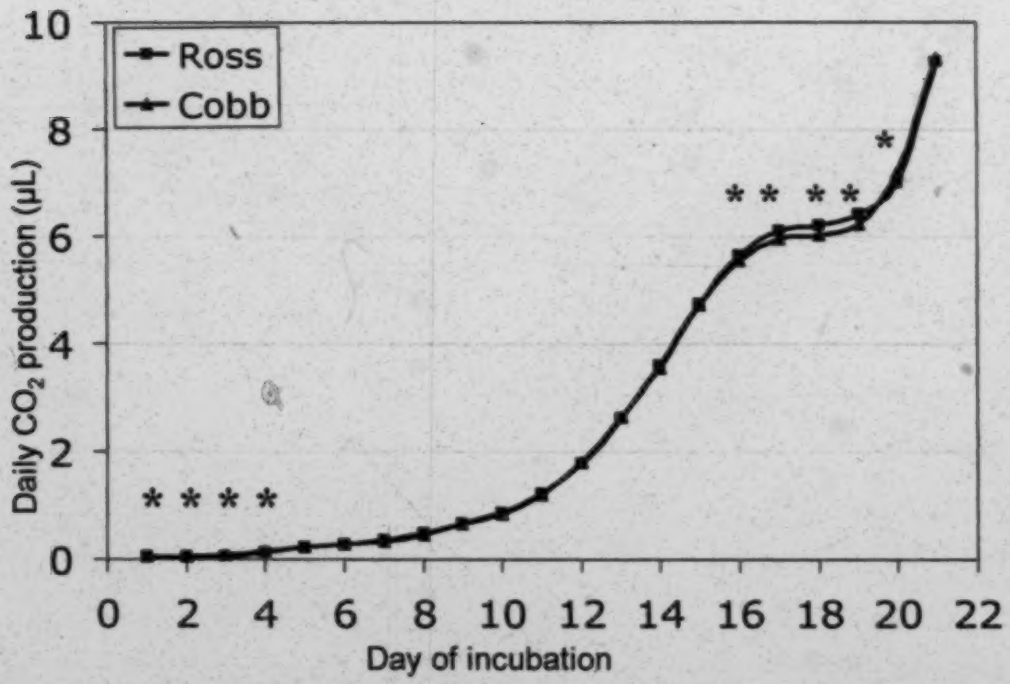

Figure 5: Effect of breeder strain on average daily embryonic CO_2 production (µL) over 21 d incubation period (* indicates significance difference between the two strains ($P \leq 0.05$)).

Table 8: Effect of breeder flock age on average daily embryonic O_2 consumption (μL) over 21 d incubation period.

Days	Young (29 wk)	Peak (34 or 36 wk)	Post peak (40 wk)	Mature (45 wk)	Old (55 wk)	VOld (59 wk)
1	0.03^{d}	0.01^{e}	0.058^{bc}	0.04^{c}	0.06^{ab}	0.07^{a}
2	0.04^{b}	0.04^{b}	0.05^{b}	0.06^{b}	0.04^{b}	0.13^{a}
3	0.04^{d}	0.04^{d}	0.08^{b}	0.05^{cd}	0.06^{c}	0.16^{a}
4	0.08^{c}	0.10^{bc}	0.17^{a}	0.11^{b}	0.17^{a}	0.18^{a}
5	0.23^{c}	0.21^{c}	0.27^{b}	0.17^{d}	0.29^{c}	0.35^{a}
6	0.35^{c}	0.33^{c}	0.33^{c}	0.18^{d}	0.86^{b}	0.66^{a}
7	0.49^{a}	0.48^{a}	0.43^{ab}	0.32^{c}	0.50^{a}	0.38^{bc}
8	0.71^{a}	0.68^{a}	0.60^{a}	0.45^{a}	0.61^{a}	1.14^{a}
9	0.93^{a}	0.97^{a}	0.96^{a}	0.77^{b}	0.92^{a}	0.91^{a}
10	1.25^{c}	1.41^{b}	1.30^{c}	1.08^{d}	1.38^{d}	1.75^{a}
11	1.85^{b}	1.97^{b}	1.88^{b}	1.57^{a}	1.96^{b}	2.42^{a}
12	2.95^{a}	2.53^{b}	2.90^{a}	2.37^{b}	2.82^{a}	3.12^{a}
13	4.11^{a}	3.68^{b}	4.14^{a}	3.49^{b}	4.09^{a}	3.73^{b}
14	6.04^{a}	6.00^{a}	5.49^{b}	4.90^{c}	6.17^{a}	5.63^{b}
15	7.78^{a}	7.20^{b}	7.06^{b}	7.09^{b}	7.94^{a}	6.93^{b}
16	9.24^{a}	8.48^{b}	8.43^{b}	8.44^{b}	9.24^{a}	8.54^{b}
17	8.70^{d}	10.24^{a}	9.02^{c}	8.81^{cd}	9.63^{b}	9.79^{b}
18	9.46^{b}	8.34^{b}	9.21^{b}	8.96^{b}	9.73^{b}	12.51^{a}
19	9.558^{c}	7.83^{d}	9.35^{c}	9.30^{c}	10.00^{ab}	10.42^{a}
20	10.07^{b}	10.84^{ab}	10.72^{ab}	10.93^{ab}	11.49^{a}	11.14^{ab}
21	14.69^{b}	10.51^{d}	13.37^{c}	14.42^{bc}	15.55^{a}	14.08^{bc}

$^{a-d}$, columnar means with different superscripts differ significantly ($P \leq 0.05$).

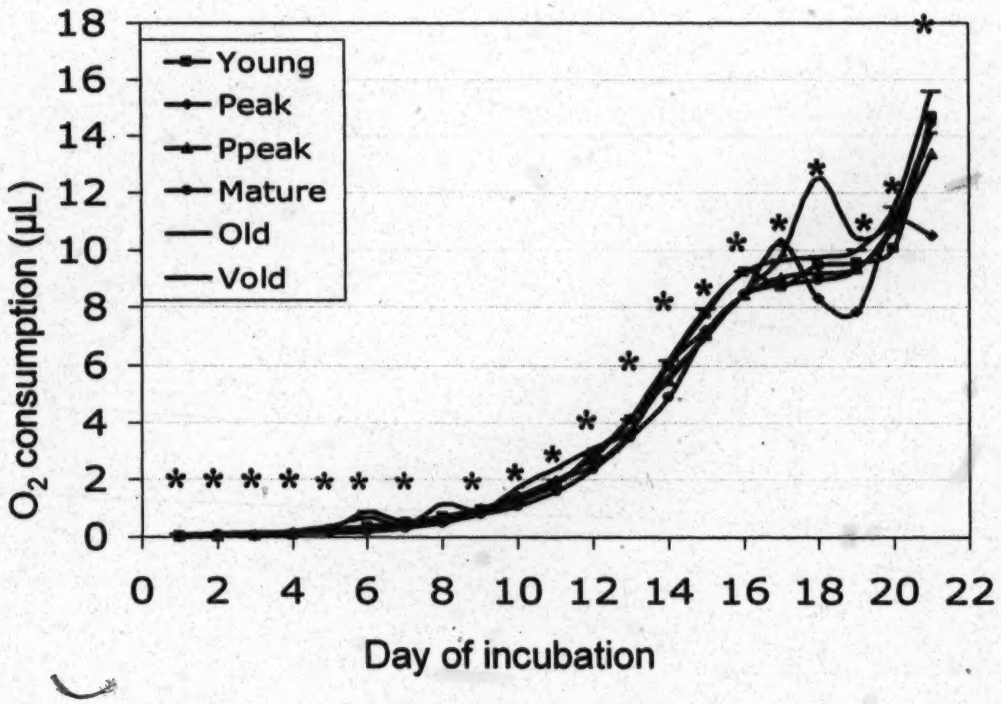

Figure 6: Effect of breeder age on average daily embryonic O_2 consumption over 21 d of incubation period (* indicates significance differences due to flock age ($P \leq 0.05$)).

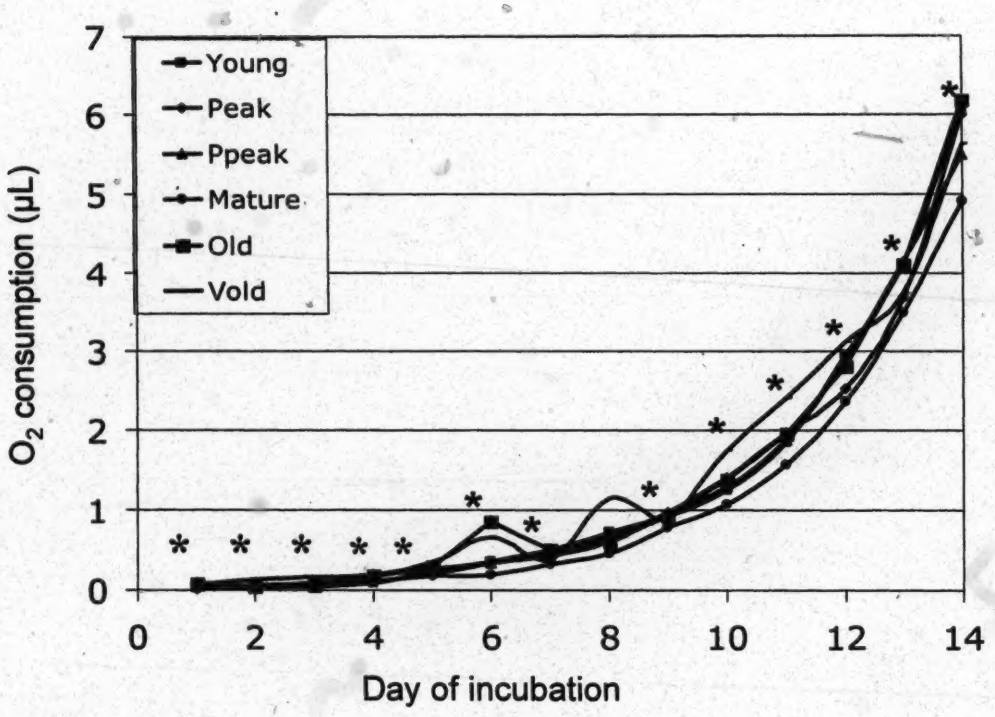

Figure 7: Effect of breeder age on average daily embryonic O_2 consumption from 0 to 14 d of incubation (* indicates significance differences due to flock age ($P \leq 0.05$)).

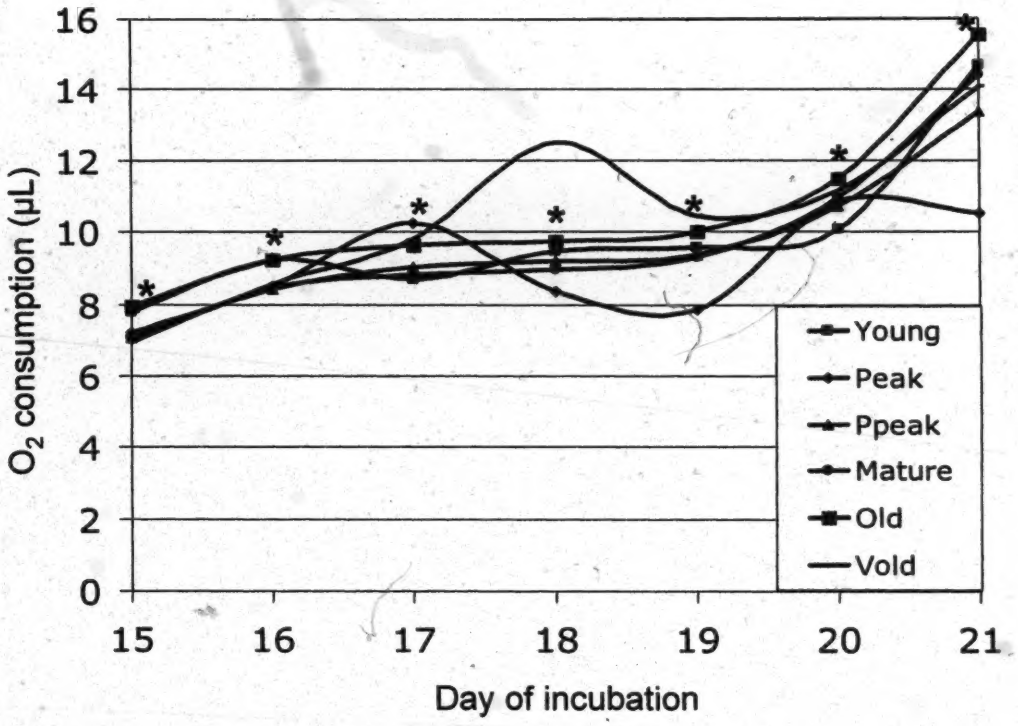

Figure 8: Effect of breeder age on average daily embryonic O_2 consumption from 15 to 21 d of incubation (* indicates significance differences due to flock age ($P \leq 0.05$)).

Table 9: Effect of breeder flock age on average daily embryonic CO_2 production (μL/s) over 21 d incubation period

Days	Young (29 wk)	Peak (34 or 36 wk)	Post peak (40 wk)	Mature (45 wk)	Old (55 wk)	VOld (59 wk)
1	0.04^{c}	0.05^{b}	0.06^{b}	0.05^{b}	0.05^{b}	0.07^{a}
2	0.05^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0.03^{b}	0.04^{a}
3	0.06^{b}	0.069^{b}	0.07^{a}	0.07^{ab}	0.05^{c}	0.06^{b}
4	0.12^{c}	0.13^{b}	0.15^{a}	0.14^{ab}	0.14^{ab}	0.14^{ab}
5	0.20^{c}	0.22^{b}	0.24^{a}	0.23^{a}	0.23^{a}	0.23^{a}
6	0.24^{d}	0.25^{cd}	0.28^{b}	$0.27b^{c}$	0.30^{a}	0.27^{b}
7	0.33^{a}	0.33^{a}	0.36^{a}	0.33^{a}	0.33^{a}	0.31^{a}
8	0.42^{c}	0.47^{ab}	0.49^{a}	0.46^{b}	0.45^{b}	0.49^{a}
9	0.58^{a}	0.77^{a}	0.69^{a}	0.62^{a}	0.59^{a}	0.69^{a}
10	0.79^{c}	0.87^{b}	0.93^{a}	0.85^{b}	0.81^{c}	0.81^{c}
11	1.11^{d}	1.24^{b}	1.31^{a}	1.22^{bc}	1.17^{cd}	1.13^{d}
12	1.75^{ab}	1.84^{ab}	1.95^{a}	1.80^{ab}	1.73^{ab}	1.62^{b}
13	2.62^{ab}	2.54^{b}	2.80^{a}	2.69^{ab}	2.49^{b}	2.54^{b}
14	3.53^{b}	3.20^{c}	3.86^{a}	3.67^{ab}	3.64^{ab}	3.52^{b}
15	4.58^{c}	4.10^{d}	4.92^{b}	5.32^{a}	4.85^{b}	4.59^{c}
16	5.50^{c}	5.11^{d}	5.64^{b}	6.43^{a}	5.68^{b}	5.12^{d}
17	5.85^{b}	5.95^{b}	6.02^{b}	6.78^{a}	5.90^{b}	5.59^{c}
18	5.76^{cd}	6.33^{b}	6.19^{b}	6.70^{a}	5.91^{c}	5.73^{d}
19	5.95^{c}	6.48^{b}	6.35^{b}	6.95^{a}	6.11^{c}	6.06^{c}
20	6.32^{c}	6.35^{c}	7.37^{b}	8.35^{a}	6.98^{b}	7.20^{b}
21	9.13^{b}	7.25^{c}	9.65^{b}	11.01^{a}	9.71^{b}	9.09^{b}

$^{a-d}$, columnar means with different superscripts differ significantly ($P \leq 0.05$).

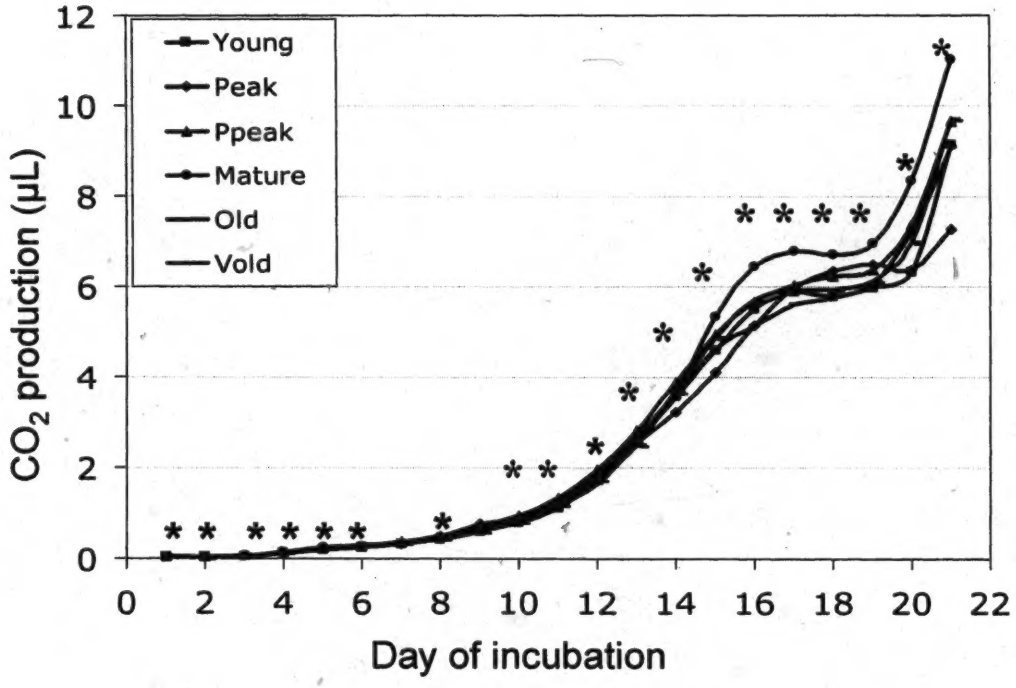

Figure 9: Effect of breeder age on average daily embryonic CO_2 production over 21 d of incubation period (* indicates significance differences due to flock age ($P \leq 0.05$)).

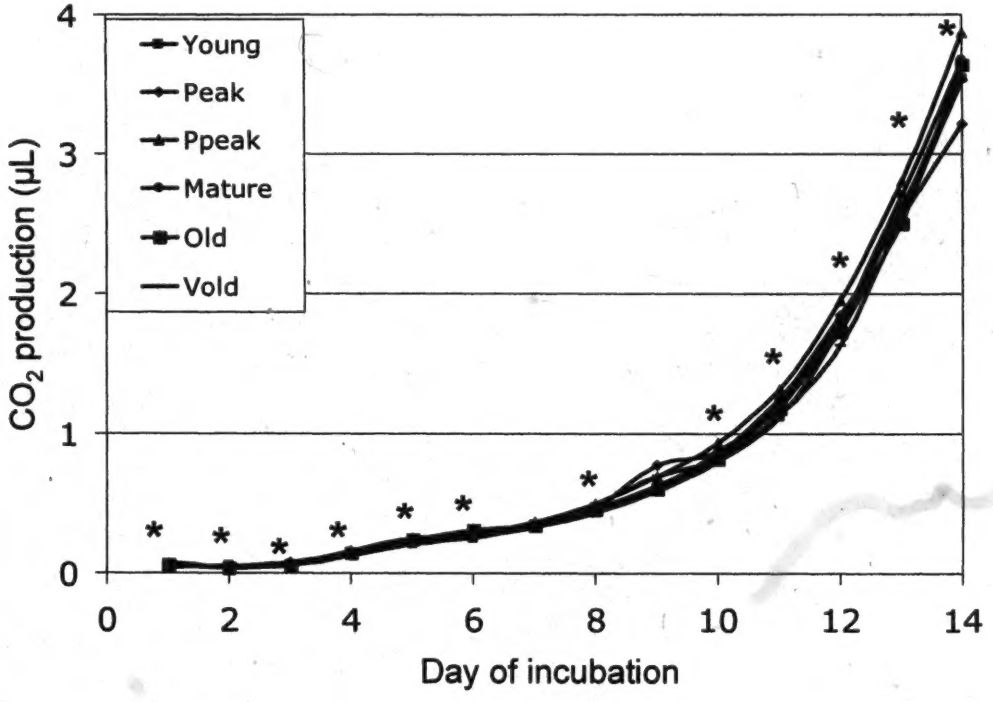

Figure 10: Effect of breeder age on average daily embryonic CO_2 production from 0 to 15 d of incubation period (* indicates significance differences due to flock age (*P≤0.05)*).

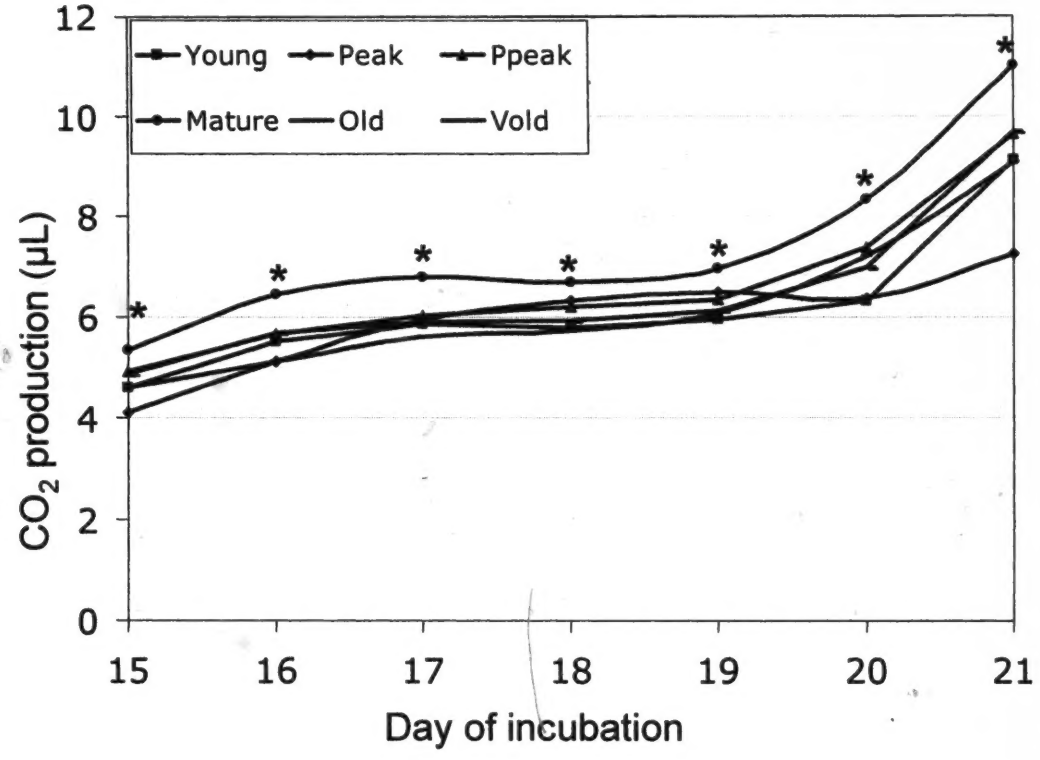

Figure 11: Effect of breeder age on average daily embryonic CO_2 production from 15 to 21 d of incubation period (* indicates significance differences due to flock age ($P \leq 0.05$)).

Table 10: Effect of two broiler breeder genetic strains and six breeder flock ages on average eggshell temperature, average embryonic heat production and total heat production over 21 d incubation period.

Source	Average Eggshell temperature [1] (^{0}C)	Average Embryonic Heat production [2] (mW)	Total Embryonic heat Production over 21 d [3] (J)
n [4]	120	120	120
Genetic strain			
Ross	38.29 ± 0.004 [a]	84 ± 1.19 [a]	1.76 [a]
Cobb	38.29 ± 0.004 [a]	82 ± 1.20 [a]	1.72 [a]
Flock age			
Young	38.23 ± 0.008 [b]	88 ± 2.14 [a]	1.85 [a]
Peak	38.30 ± 0.007 [a]	75 ± 2.30 [b]	1.58 [b]
Post peak	38.31 ± 0.007 [a]	76 ± 1.95 [b]	1.60 [b]
Mature	38.31 ± 0.007 [a]	85 ± 2.06 [a]	1.79 [a]
Old	38.30 ± 0.007 [a]	85 ± 1.96 [a]	1.79 [a]
Very Old	38.31 ± 0.007 [a]	89 ± 1.98 [a]	1.87 [a]
Interactions			
Ross * Young	38.24 ± 0.011 [c]	90 ± 2.88 [a]	1.89 [a]
Ross * Peak	38.27 ± 0.010 [b]	74 ± 3.18 [a]	1.55 [a]
Ross * Post peak	38.32 ± 0.011 [a]	75 ± 2.84 [a]	1.58 [a]
Ross * Mature	38.32 ± 0.010 [a]	86 ± 2.91 [a]	1.81 [a]
Ross * Old	38.31 ± 0.010 [a]	88 ± 2.85 [a]	1.85 [a]
Ross * Very Old	38.32 ± 0.010 [a]	91 ± 2.78 [a]	1.91 [a]
Cobb * Young	38.23 ± 0.011 [c]	87 ± 3.15 [a]	1.83 [a]
Cobb * Peak	38.34 ± 0.011 [a]	75 ± 3.33 [a]	1.58 [a]
Cobb * Post peak	38.29 ± 0.010 [a]	77 ± 2.67 [a]	1.62 [a]
Cobb * Mature	38.30 ± 0.010 [a]	83 ± 2.93 [a]	1.74 [a]
Cobb * Old	38.29 ± 0.010 [a]	82 ± 2.69 [a]	1.72 [a]
Cobb * Very Old	38.31 ± 0.010 [a]	86 ± 2.83 [a]	1.81 [a]

[a-b], columnar means with different superscripts differ significantly ($P \leq 0.05$.)

[1] Average eggshell temperature = Mean of all daily eggshell temperature recorded / 21 d of incubation.

[2] Average Embryonic Heat production = [3.871 x CO_2 consumption (L/d) + 1.194 x CO_2 production (L/d)] x 1 d / 24h x 1 h / 3600s x 1000cal / 1 kcal x 4.187 J / cal x 1000 mW/W

[3] Total Embryonic Heat production = Sum of average daily embryonic heat production over the 21 d incubation

[4] n = Initial number of experimental eggs incubated

Table 11: Effect of two broiler breeder genetic strains on average daily embryonic heat production (mW).

Days	Ross 308	Cobb 500
1	1.03[a]	1.17[a]
2	1.19[a]	1.30[a]
3	1.49[a]	1.64[a]
4	2.89[b]	3.08[a]
5	5.25[a]	5.32[a]
6	8.77[a]	8.76[a]
7	9.27[a]	8.39[b a]
8	12.61[a]	14.51[a]
9	18.30[a]	17.76[a]
10	26.47[a]	26.08[a]
11	37.64[a]	37.17[a]
12	53.64[a]	54.54[a]
13	76.52[a]	75.08[a]
14	112.74[a]	110.80[a]
15	143.13[a]	142.88[a]
16	172.14[a]	167.38[b]
17	183.62[a]	180.20[b]
18	194.96[a]	180.78[b]
19	181.30[b]	186.58[a]
20	212.11[a]	218.96[a]
21	272.90[a]	269.91[a]

[a-b], columnar means with different superscripts differ significantly ($P \leq 0.05$).

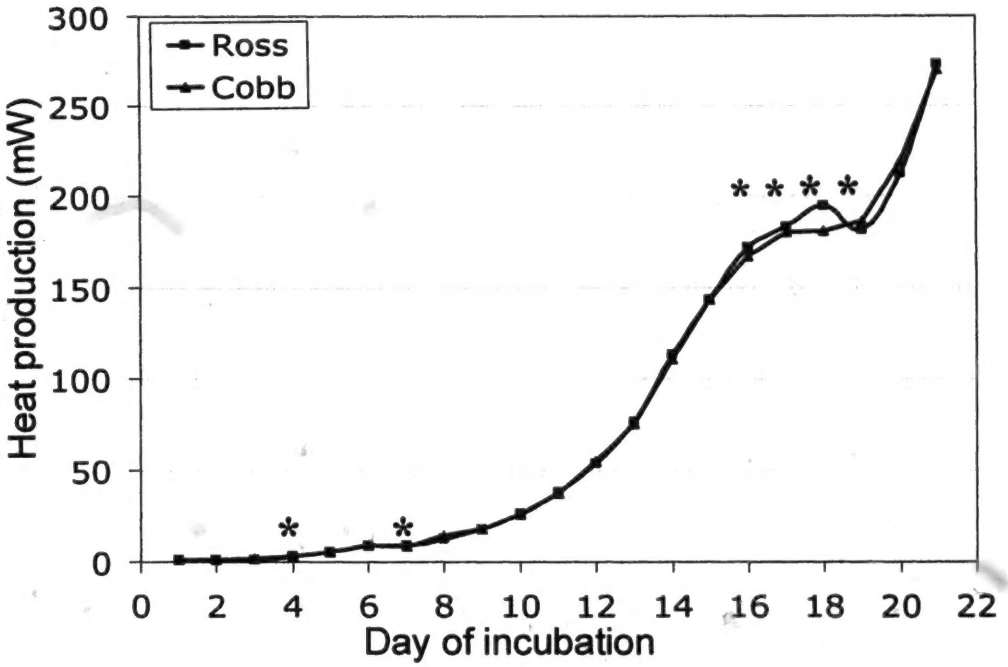

Figure 12: Effect of breeder strain on average daily embryonic heat production (mW) over 21 d of incubation period (* indicates significant difference between two strains).

Table 12: Effect of breeder flock age on average daily embryonic heat production (mW) over 21 d of incubation.

Days	Young (29 wk)	Peak (34 or 36 wk)	Post peak (40 wk)	Mature (45 wk)	Old (55 wk)	VOld (59 wk)
1	0.67^{c}	0.52^{c}	1.27^{ab}	1.09^{b}	1.41^{ab}	1.63^{a}
2	0.91^{b}	0.99^{b}	1.11^{b}	1.21^{b}	0.90b	2.36^{a}
3	1.06^{4}	1.12^{c}	1.68^{b}	1.32^{c}	1.32^{c}	2.90^{a}
4	1.97^{c}	2.314^{c}	3.53^{a}	2.61^{b}	3.58^{a}	3.90^{a}
5	4.87^{c}	4.49^{c}	5.59^{b}	3.92^{d}	5.99^{b}	6.85^{a}
6	7.13^{c}	6.67^{c}	6.83^{c}	4.37^{d}	15.46^{a}	12.12^{b}
7	9.82^{a}	9.42^{a}	8.81^{ab}	6.93^{c}	9.92^{b}	8.07^{b}
8	13.76^{a}	13.40^{a}	12.30^{a}	9.65^{a}	12.17^{a}	21.86^{a}
9	18.03^{a}	18.97^{a}	19.19^{a}	15.67^{b}	18.02^{a}	18.30^{a}
10	24.33^{c}	27.18^{b}	25.77^{bc}	21.78^{d}	26.53^{b}	32.06^{a}
11	35.62^{b}	38.11^{b}	37.18^{b}	31.45^{c}	37.10^{b}	44.95^{a}
12	57.60^{a}	50.24^{b}	56.76^{a}	47.39^{b}	54.36^{a}	58.20^{a}
13	78.54^{a}	70.06^{b}	81.21^{a}	69.95^{b}	78.82^{a}	76.21^{ab}
14	115.74^{ab}	121.44^{a}	108.39^{b}	97.88^{c}	118.25^{a}	108.92^{a}
15	149.51^{a}	138.61^{b}	139.17^{b}	141.52^{b}	152.98^{a}	136.25^{b}
16	177.31^{a}	163.28^{c}	164.86^{c}	171.02^{b}	178.21^{a}	163.91^{c}
17	170.33^{d}	195.87^{a}	176.45^{c}	176.80^{c}	185.72^{b}	186.30^{b}
18	182.35^{bc}	167.03^{d}	180.18^{c}	178.77^{c}	187.43^{b}	231.45^{a}
19	184.95^{b}	157.72^{c}	183.42^{b}	185.59^{b}	192.65^{ab}	199.32^{a}
20	194.74^{c}	207.26^{b}	210.96^{b}	219.16^{b}	221.17^{b}	239.91^{a}
21	282.74^{b}	205.94^{c}	268.64^{b}	288.56^{b}	306.21^{a}	276.31^{b}

$^{a-d}$, columnar means with different superscripts differ significantly ($P \leq 0.05$).

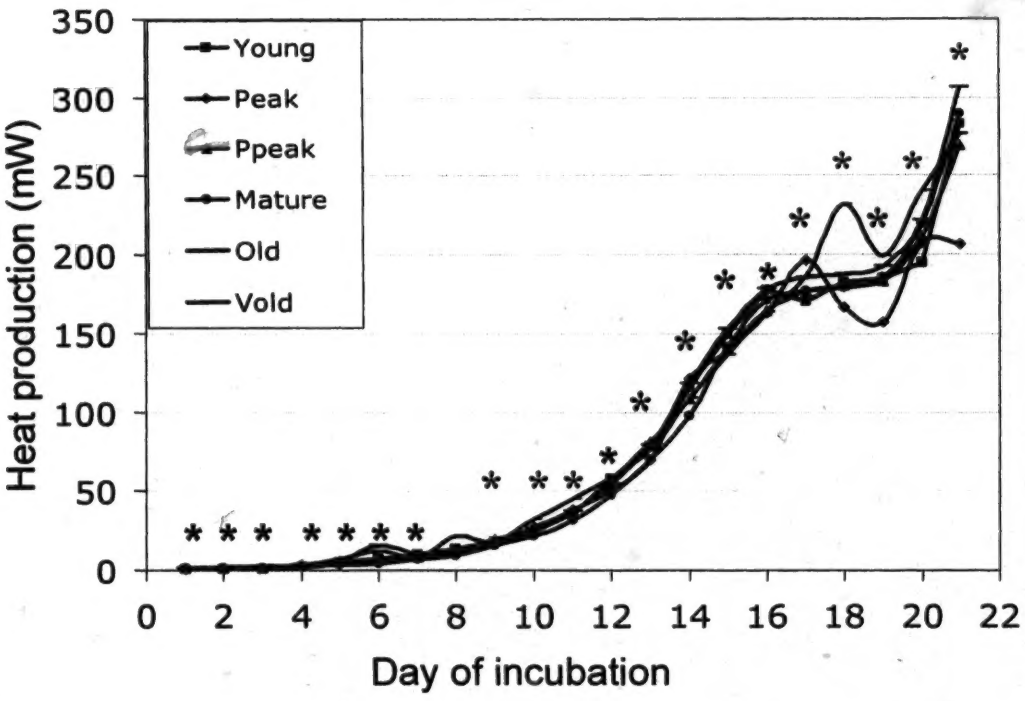

Figure 13: Effect of breeder flock age on average daily embryonic heat production (mW) over 21 d of incubation (* indicates significant difference due to flock age).

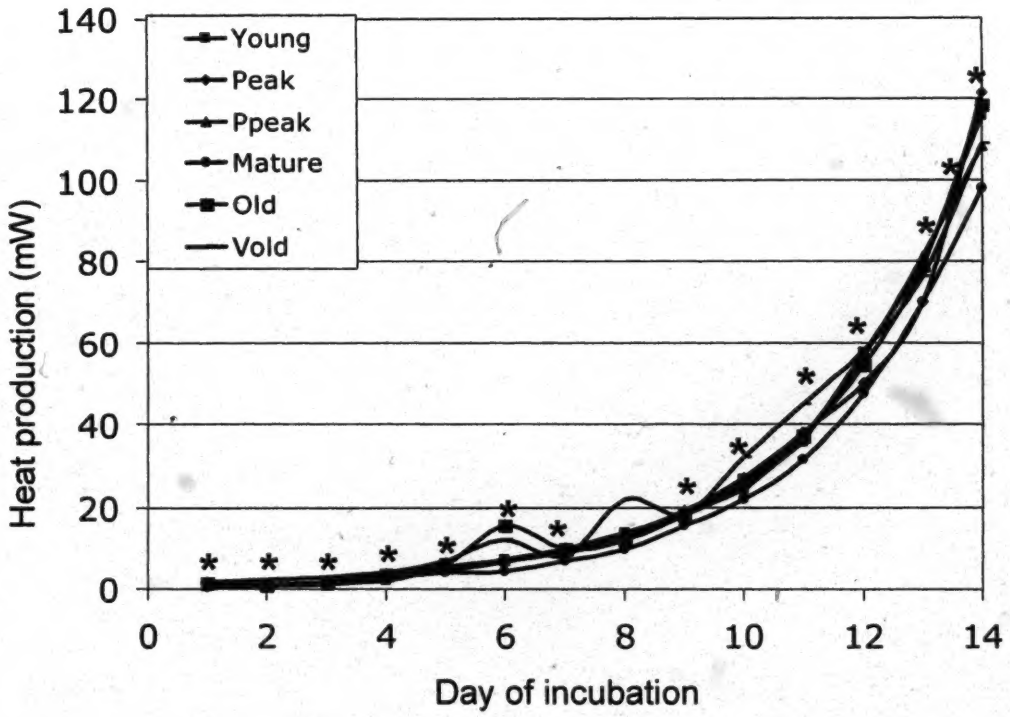

Figure 14: Effect of breeder age on average daily embryonic heat production (mW) from 0 to 14 d of incubation (* indicates significant difference due to flock age).

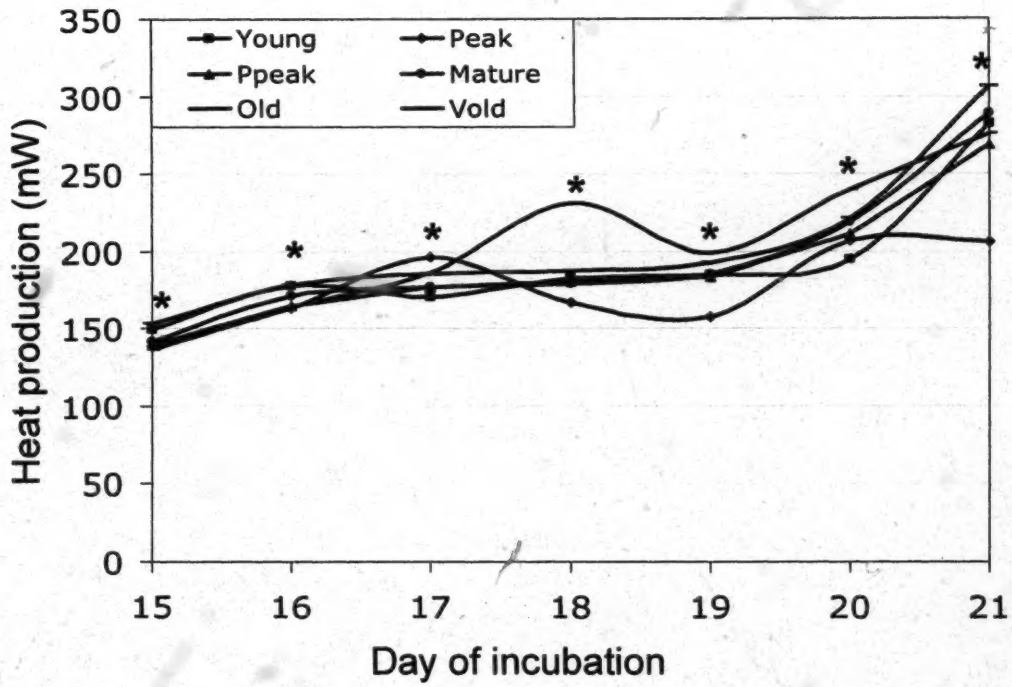

Figure 15: Effect of breeder age on average daily embryonic heat production from 15 to 21 d of incubation (* indicates significant difference due to flock age).

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CHAPTER 4: GENERAL DISCUSSION AND CONCLUSION

Avian embryonic metabolism has long been a subject of biological interest. It is also a problem of obvious economic importance. The total embryonic mortality of domestic fowl, *Gallus gallus*, has been progressively increasing over time. In past years (30 yr ago) when flocks were small the embryonic death rate rarely exceeded 10% (Boerjan, 2004). But as small scale and simple methods of poultry production have been replaced by large scale modern practices aimed at intensive production and large scale artificial incubation of eggs, embryonic mortality has frequently risen to 25% or more over the entire length of incubation (Romanoff, 1949). Numerous investigations have attempted to determine the causes of death during the incubation period, and to explain the rising embryonic death rate as the modern industry has advanced. In chicken embryos this may be linked with embryonic metabolism and genetic selection. Some studies have indicated that modern genetics and selection for rapid embryonic growth has cause metabolic imbalances and reduced O_2 transfer between the embryo and its immediate environment (Julian, 1998).

Developing embryos require a constant influx of oxygen for respiration and an associated removal of CO_2 from the egg. However during late embryonic development when embryonic O_2 requirements exceed the O_2 supply that can be delivered via simple diffusion across the eggshell, this hypoxic state can retard embryo growth; this includes development of important organs such as the heart (Christensen et al., 2003). The eggshell thus becomes an important factor determining gas exchange.

An attempt by Meir and Tawazza (1999) to increase shell gas diffusion by drilling extra holes showed that though O_2 diffusion increased with increased number of

holes, it was still not sufficient to compensate fully for the low O_2 availability caused by low eggshell conductance which is a characteristic of certain species of birds. This account shows that selection may have hindered the ability of the growing embryo from breathing well because of limitation put on the eggshell relative to the size of the embryo. The limitation of gas exchange between the embryo and its environment, can affect the rate of embryonic metabolism within the embryo. Since embryos acquire about 90% of their energy from metabolism of yolk (ß-oxidation) there is a need for sufficient O_2 to continue the process until the chick is hatched (Murray, 1925).

In rapidly growing embryos, from 18 d of incubation onwards embryonic O_2 requirements cannot be met by eggshell diffusion. If these embryos also have a higher level of metabolic heat production they not only have to cope with hypoxia, but stress of overheating due to rapid growth incurred due to genetic selection (Feast et al., 1998). It has therefore become important that excess metabolic heat be removed to reduce the risk of overheating (A. A. McCready (Maple Leaf Poultry, Wetaskiwin, Alberta, personal communication).

Because of the above it is important to understand the relationship that exists between eggshell conductance and embryonic metabolism. Since eggshell conductance restricts the movement of gases across the eggshell, this can have an influence on embryonic metabolism. In experiment 1, the data showed that eggshell conductance was not affected by either genetic strain or flock age. However, it is important to note that egg size was kept equal irrespective of strain or flock age. In experiment 2, genetic strain did not affect average or total embryonic metabolism over the entire 21 d of incubation. However, daily effects of genetic strain on embryonic metabolism were

observed. Parent flock age significantly influenced both average and total embryonic metabolism as well as metabolism during particular days of incubation; in general, embryos of older parent flocks had higher metabolism compared to younger flocks.

It may be important that as embryonic metabolism increases, either by the influence of genetic strain or parent flock age, that the eggshell conductance increase as well so that sufficient O_2 by embryo is obtained (Poultry Industry Council, 2006). If the demand for O_2 of the embryo (metabolism) exceeds the supply of O_2 (eggshell conductance), it can affect embryonic survival and organ growth.

The current research shows that eggshell conductance has not increased even though embryonic metabolism has been altered. It is likely that embryo mortality that occurred in these strains and flock ages is the result of the absence of change in eggshell conductance as embryo metabolism increases with flock age. This is especially important during the latter stages of incubation when the embryo is attempting to exit the shell. Though data for embryonic mortality was not shown for the present study (due to relatively small number of eggs used in experiments) most embryonic deaths in the experimental eggs occurred between 17 and 21 d. This is the period in which embryonic metabolism can exceed the supply of O_2.

The results of this research provide evidence that, in order to maximize embryonic survival, incubation conditions may have to be developed specifically for individual genetic strains and flock ages. In addition, it should be important to test the effect of egg size on metabolism at different flock ages and in different strains.

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APPENDIX: Set up of Metabolism Equipment

Set up of the two vacuum manifolds involved a system where the hardware components of a respiratory gas exchange systems are connected. The set up means that while sampling air through the whole metabolism equipment, changes in CO_2 and O_2 concentrations due to respiration is monitored continuously and not in separate pathways. This system is called the open flow mode. The entire metabolism equipment was arranged as described below.

First, the incubator is connected to the CO2/H2O analyzer via the solenoid valves. Then the CO_2/H_2O analyzer is connected to the DOX. Between the CO_2/H_2O analyzer and the DOX, a constant flow sub-sampling pump was used to draw air through the CO_2/H_2O analyzer which releases it to the DOX sample inlet. The sub-sampling pump checked by a gas controller monitor dictates gas flow through the pump to a constant rate of 150 or 300ml/min or as required. The Dry cal was attached daily to the inlet of the sub-sampling pump to calibrate it. This pump was adjusted daily by the Dry cal and its needle to a flow rate of 150 or 300ml/min or as required. Only 25 psi/25mL/min of air pulled from the CO_2/H_2O analyzer enters into the DOX. (During calibration 25mL/min or air drawn in was take to be equivalent to 25 psi). Air through the DOX was operated by two DOX controller pumps. One pump was used to obtain air from the air sample going thorough the CO_2/H_2O analyzer. The other pump pulled a sample of air coming directly from the incubator. The pumps were set to pull only 25 ml/min or psi of air through each of the DOX inlets ports. In this way a zero differential pressure between the sample and reference cells of the DOX can be attained to prevent damage to the DOX active cells.

To ensure that changes in atmospheric pressure does not drift the differential pressure from zero and disturb O_2 readings, both inlet ports of the DOX were fitted with needle valves which were adjusted daily to bring differential pressure to zero. The DOX sample cells can easily be damaged by moisture, so syringe tubes containing anhydrous magnesium perchlorate[1] and drying tubes were connected to the inlet of all pumps leading to the DOX to absorb the moisture. In addition, the inlet ports of the DOX were fitted with drying columns filled with anhydrous magnesium perchlorate to prevent damage from moisture. All these restricted excess air flow through the DOX which damage its cells. Finally the air leaves the system through the DOX gas controller pump into atmosphere

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